Coronavirus / Corona Virus = Non Existing , Unproven Political Virus .

PCR the ultimate in virus identification tests. Actually a joke , a hoax , a scam , a ploy , a fraud. A "sample" is collected from a sick person. Who decided the blood or spinal fluid etc will have the sickness causing virus ? Even if something can be identified and isolated , it represents the result / the effect of that sickness . It doesn't represent the cause . Even if that particular something is capable of causing sickness in another healthy person , it doesn't represent the cause of the sickness . Nothing is identified & isolated from from a pure raw sample . HIV the most studied virus , till date is fiction , fabrication , fraud .No scientific paper available proving the existence of any such virus .

Coronavirus & HIV are retroviruses . They decided so . Without any basis . It was called RNA virus , later renamed retrovirus . Purely theoretical , assumptions , fraudulent claims . They climed something to be reverse transcriptase enzyme . They add that to the sample and they claim the RNA has transcribed into DNA . for a change they call it cDNA. DNA has a double helix structure . Based on findings of theoretical scientists Francis Crick & James Watson , freemasons who were into group sex

orgies and late night parties . They supposedly stole it from the findings of another non scientist Rosalind Franklin from Rothschild family , whose whereabouts cannot be verified deeply.
They heat the so called sample many times , add many chemicals , plant hormones , infected animal materials , centrifuge , filter to get purified / isolated virus . Add dyes , flouroscents .
Then they keep this under a fancy property called Electron Microscope and get some weired picture on the computer screen and claim it to be the sickness causing virus . EM can , if at all , identify only inanimate substances , according to their own admissions .

Electron is fiction . Atom is fiction. Molecule is fiction . Atomic number / Molecular weight is fiction . Its atomism . Theosophy . Occult Chemistry .
Results of EM , not verifiable . Its theoretical science . Pure fantasy or rather Political Conspiracy .

Human body is not made up of Cells. DNA /Gene / Chromosomes – Unproven science .

Antibody test is superior fantsy . fraud .
Conspiracy .They claim to test for the antibody produced by body against unproven virus .

All claims , with no scientific backing .

Koch Postulates . Postulates means Assumptions , Suggestions , Beliefs. Robert Koch . Hitler called himself Robert Koch of Politics . RK played a prominent role in Nazi Germany . Koch Brothers funded Hitler . From same family ? Must be .

Any virus is unproven . Unfounded .
The below material is subject to above points.

Coronavirus is a single word . other so called viruses are 2 words.

Italy is supposed to have better healthcare facilities and recording practices than China. We don't find Italy to be having either. Figures given out are totally unreliable , not genuine .

We don't have conclusive evidence covid 19 or any virus exist .

Different people react differently to seasonal changes in climate . its not Virus .
Then there is poison - in medicine , food , water , atmosphere. Its not virus .

In the **winter seasons** from 2013/14 to 2016/17, an estimated average of 5,290,000 ILI - **influenza like illness –** (note **"like"**) cases occurred in Italy, corresponding to an incidence of 9%. • More than 68,000 deaths **attributable** to flu epidemics were **estimated** in the study period. • **Italy** showed a higher influenza attributable excess mortality compared to **other** European countries. especially in the **elderly**.
In **recent** years, **Italy** has been registering **peaks** in death rates, particularly among the **elderly** during the **winter** season. **"Influenza"** epidemics have been indicated as **one of the** potential determinants of such an excess. The objective of our study was to estimate the influenza-attributable contribution to excess mortality during the influenza **seasons** from 2013/14 to 2016/17 in Italy.

Estimated **excess** deaths of 7,027,-- 20,259,-- 15,801 and 24,981 attributable to influenza epidemics in the 2013/14, 2014/15, 2015/16 and 2016/17, respectively. The **average** annual mortality **excess** rate per 100,000 ranged from 11.6 to 41.2 with most of the influenza-associated deaths per year registered among the elderly. However **children less than 5 years old also** reported a relevant influenza attributable **excess** death rate in the 2014/15 and 2016/17 seasons (1.05/100,000 and 1.54/100,000 respectively).

Over 68,000 deaths were attributable to influenza epidemics in the study period. The observed excess of

deaths is **not** completely **unexpected**, **given the high number of fragile very old subjects living in Italy.**

Seasonal influenza epidemics make a substantial contribution to the worldwide annual mortality rate, in particular among **elderly** individuals aged 65 years and over. Influenza associated deaths are **highly variable by country and season** (Iuliano et al., 2018).
Factors influencing this variability may include **environmental temperature;** and population demographics (e.g., the **proportion** of elderly individuals and/or of individuals with **chronic** conditions).
During the winter seasons 2014/15 and 2016/17, an excess of all-cause mortality was reported in Europe.

In recent years, **Italy** has been registering **peaks** in death rates, particularly among the **elderly** , **during the winter season.** A mortality rate of 10.7 per 1,000 inhabitants was observed in the winter season 2014/2015 (more than 375,000 deaths in absolute terms), corresponding to an estimated 54,000 **excess** deaths (+9.1%) as compared to 2014, representing the **highest** reported mortality rate since the Second World War in Italy.

Excess mortality for influenza in Italy in the above mentioned seasons has been previously explored in a multi-country study, analysing mortality data from a

limited sample of the Italian population, and in a study focusing on a **single** Italian **region**.

The present study aims to investigate the two mortality **peaks** observed in Italy during 2015 and 2017, using the following data: a) census mortality data from **all** causes from 2013 to 2017; b) seasonal influenza **like**-illness surveillance data from 2013/14 to 2016/17 (week 42 to week 17); c) environmental temperature data for the same years.

The final objective was to estimate the "influenza"-**attributable** deaths and the contribution of **temperature** variation to the excess mortality during the above mentioned influenza seasons, using a **multiplicative Poisson regression model**.**??????** (simulation as always ?????).

More than one hundred Italian weather stations contribute to the NOAA database, providing daily average, minimum and maximum temperatures. Overall, Italian daily average, minimum and maximum temperatures were obtained computing the means of daily average, minimum temperatures and maximum temperatures from each weather station, weighted by the populations of the Italian provinces where the stations were located for all of the study period (winter seasons from 2013/14 to 2016/17). Weekly average temperatures

as well as weekly minimum and maximum temperatures were obtained calculating the weekly average of daily average, minimum and maximum temperatures. Based on these overall weekly temperatures, we estimated the expected weekly minimum and maximum temperature **using a general linear model** with a yearly seasonal variation applied to the data of the entire study period. Weeks with **extreme** temperatures (EC) were defined as weeks with an average temperature above the average of the maximum weekly temperatures or lower than the average of the minimum weekly temperatures.

Statistical analysis . (???) .The number of influenza-attributable deaths was **estimated** using the FluMOMO algorithm, based on the weekly Influenza Activity (IA) and ET (EuroMOMO, 2018b). For this analysis, we used two IA indicators: 1) the ILI incidence and 2) the Goldstein index (ILI × percentage of positive specimens) (Goldstein et al., 2011). Up to two-weeks-delayed effects of the explanatory variables were considered in the model. An explanatory factor reflecting the deviation of environmental temperature from the average maximum/minimum temperatures was introduced in the model in order to take into account a potential confounding effect of temperature on influenza excess mortality, **as many Italian regions are affected by very cold weather in some winter weeks** (e.g. January 2017).

Very cold weather is recognized to have a potential impact on the excess mortality from all causes (Nielsen et al., 2011). Therefore, we estimated the influenza-attributable deaths among older adults, **adjusting** for Extreme Temperatures (ET), defined as weeks with a mean temperature above the average maximum temperature or below the average. **Periods with excess cold might be bad in the winter, but in summer, it may have a benign effect and opposite for periods with excess warmth.**

Therefore, the winter effect of temperature is included with an **opposite warm (protective)** and **cold (harmful)** effect. The method has been described elsewhere (Vestergaard et al., 2017). In brief, we adopted a Poisson regression time-series **model** (simulation again) with over-dispersion, where the weekly absolute number of deaths from **all** causes was the outcome variable and IA and ET the explanatory variables. In the results section we reported results including both **models** with and without the ET effect. We **corrected** the model by annual trend, and seasonality. Seasonality was expressed as the sum of two **sine** waves of one year and half year periods, respectively (Nielsen et al., 2018).

Analyses were performed separately for the age groups 0–4, 5–14, 15–64 and 65+ years of age, as well as for all ages. The **statistical** analysis was performed using STATA version 14 (StataCorp, 2014).

Results … National deaths … **A total of 1,457,038 deaths were registered in Italy during the study period.** 2013 /14 to 20116/17

Table 1 provides the **absolute** number of **all**-cause deaths, the overall crude mortality rate (per 1,000 inhabitants), the overall standardized mortality rate (per 1,000 inhabitants) **and** the standardized mortality rate by age group and by season. The number of deaths and the mortality rates from **all** causes **increased by age.** The 2014/15 and 2016/17 seasons showed the **highest** overall crude and standardized mortality rates **(better registering ??).**

Influenza-like illness surveillance data During the study period, an average of 5,290,000 (range 4,542,000–6,299,000) ILI cases were estimated in Italy, corresponding to a cumulative average incidence of 9% (range 8%–11%) in the **Italian** population. The **highest** estimated incidence was observed **in children younger than 5 years** (average of 23%, range 21%–26%) and in **adolescents** (average of 15%, range 12%–18%). The 2014/15 season showed the **highest** estimated number of cases, with a total of 6,300,000 ILI cases. The **lowest** number of cases was observed in the 2013/14 season, with 4,540,000 ILI estimated cases (Table 2).

Influenza-attributable mortality. We observed two peaks, one for the 2014/15 and one for the 2016/17 season. These two seasons were also characterized by a high ILI incidence, **particularly** high for people aged 65 years and over (data not shown).
The **average annual** mortality excess rate (MR) ranged from 40.6 to 70.2 per 100,000. The total number of **excess** ILI-attributable deaths during the 2014/15 season was 41,066, -- 65.6% **higher** compared to the **previous** season**??**. During the 2016/17 season, the number of ILI-attributable **excess** deaths was 43,336, -- 57.9% more than the previous season**??**.
Using the Goldstein index??, the total number of **excess** deaths attributable to influenza in the 4-season study period was 68,068.

Temperature associated mortality.
Extreme temperatures .. The overall number of deaths attributable to extreme ambient temperature in the study period was 8,820, ranging from 939 during winter 2014/15 to 5,190 during winter 2016/17, corresponding to a 3.6 average MR (range: 1.5 to 8.6, data not shown) per 100,000. Discussion With the present study we show a remarkable **excess** death attributable to influenza in Italy during the **winter** seasons 2014/15 and 2016/17, which was independent from mean weekly extreme temperature variations. Our results show that during these two seasons, in Italy, a **high** proportion of deaths was

observed among the **elderly** (96.1% and 77.7%, respectively).

Scarce data is available on influenza-attributable mortality estimates for single countries in the study period considered. However, some studies have been published that have reported influenza-attributable excess mortality rates in EU countries. In particular, **Italy** shows a **higher** influenza attributable excess mortality compared to **Denmark** in all ages, with highest levels reported in **elderly**, but for the **0–4** age group where **Denmark** reported higher rates compared to **Italy** in **all** seasons, **except** for the 2014/2015 season (0.52/100,000 vs 1.05/100,000).

In the **UK**, estimates of the annual number of deaths directly attributable to influenza range from 4 to 14,000 per year, with an average of around 8,000 per year (Public Health England, 2014). Moreover, influenza-attributable **excess** deaths using the **FluMomo method??** for UK were reported in 2014/15. UK estimates, in terms of absolute numbers, were **higher** compared to **Italian** data, in **all** ages and in particular in the elderly (26,542 vs 19,475 respectively).

Plausible hypotheses regarding the determinants of the observed excess deaths attributable to influenza in Italy, especially in the old population (i.e. 65+), are: i) meteorological factors (**low and high** temperatures), ii) the amplitude of the at risk population (pools of elderly).

Deviation from expected temperature may have a great impact on mortality.
Very low temperatures were registered at the beginning of 2017 in various European countries. Therefore, we decided to **adjust** our estimates of influenza-associated mortality for **extreme** temperatures. We found that the **impact** of extreme temperatures on mortality in Italy was quite limited**??**, with the **exception??** of the 2016/17 season. Despite this impact of **extreme low** temperatures, most of the excess death rate registered in 2016/17 is attributable to influenza, confirming other observations recorded in Europe. Nevertheless, this is the **first** study reporting the **effect of temperatures on** mortality in Italy, and we acknowledge that this association **has to be further investigated, also** analyzing this factor at sub-national level.
In terms of amplitude of the at risk population, in **Italy** there are **6.7 million of people aged 75+** (more than 10% of the population) that constitute **a large group of fragile subjects, among which the annual death rate is naturally high,** around 4%. Among them, a **large** variation in the absolute number of deaths causes **small** fluctuations in the mortality rate.

This study has several limitations.

The influenza surveillance system in Italy is based on voluntary general practitioners reporting ILI cases,

and the participating general practitioners are not selected with random criteria.

Another important limitation in the surveillance system is related to “virological” surveillance because sampling of influenza testing may be **biased towards more samples taken at hospitals, and therefore may overestimate the proportion of positive samples in the population.**

These limitations may introduce a potential **bias** due to the selection of subjects under surveillance. Moreover, the study is **based** on census mortality data, while **previous** published studies were based on **sample** data and limited to regional data. However, the proposed model uses all-cause weekly mortality data, usually available quite in real time in many countries, and can therefore be a valuable tool for monitoring the seasonal impact of influenza.

The study should be **validated** using **cause specific** mortality data, which,however,was **not available** for the **entire** study period.

Furthermore, it would be valuable to investigate also regional patterns, but such details on mortality were **not available** in the study period considered. To evaluate **whether** the association of influenza activity with mortality varied with temperatures, an interaction term of

influenza activity and temperatures should be added to **model**. The adopted **statistical** model did **not** include an interaction term between temperatures and IA. This "**rigidity**" of the model can be considered a **limitation** and should be overcome in **future** applications.

Finally, the pattern of the **effect** of temperature on mortality should be investigated further to be able to obtain **more valid estimates** of the impact of this effect, e.g. testing different cut-off **values** for the extreme temperature **definition**.

Assessment of winter mortality in Italy, during the 2014/15 and 2016/17 seasons, confirmed the hypothesis that influenza was **likely** to have been the main contributor to the excess mortality seen, especially in the **elderly**.

Italy

In Europe, as luck would have it, the pandemic **first** affected northern Italy, namely **Lombardy** and **Veneto**, which have by far the largest number of vaccine **hesitant** people in Europe and probably the world. Veneto strongly opposed the expansion of vaccine mandates. Activists demonstrated for months, with rallies of more than 50,000 people. As a result, the regional government appealed to the Council of State, arguing that the law **violated constitutional** freedoms and demanded autonomy in

health matters. Of note, the **WHO** then decided to move its European headquarters to **Venice**, the **capital** of **Veneto**.

At the beginning of the disease outbreak, the Italian authorities considered it **un**necessary to impose a two-week school quarantine on children returning from a trip to **China**, in order not to "stigmatize" them. (By contrast, **un**vaccinated children are stigmatized **and** prohibited from attending school year round.) Officials **disagreed** on Covid-19 diagnosis and "crisis measures," reflecting conflicts between regional parties **and** medical experts. But the **WHO** soon managed to take **control** of the situation and appointed a special advisor, **Dr. Gualtiero Walter Ricciardi,** who had been forced to resign earlier from the Italian HHS due to **a long list of undeclared conflicts of interest,** to steer the coronavirus crisis.
Since then, panic and alarm have escalated continuously, as have the Veneto region's accusations of "anti-scientific" management. Although the country has been in a complete lockdown for weeks, cases keep increasing and the estimated number of deaths is now nearing 3,000.

This sends a frightening signal, **but these numbers need to be seen with caution.** **First**, one of the major reasons why Italy is "overwhelmed," is because of the crisis , its public hospitals were already facing before the epidemic.

The number of intensive care units has dropped by half over the last 20 years, dropping **from** the highest to the lowest number of beds per capita in Europe to around 230 per 100,000 inhabitants. In other words, the situation was **already** disastrous.

Second, there is a lot of **controversy** about the number of deaths that can really be ascribed to the epidemic. **Testing is not very reliable and suffers many biases.** According to **Dr.Wolfgang Wodarg,** who had chaired the Parliamentary Assembly of the Council of Europe Health Committee that called an emergency debate on the influence of the pharmaceutical industry in the declaration of the H1N1 flu pandemic by WHO in 2009, **"the tests are currently not measuring the incidence of coronavirus diseases, but the activity of the specialists searching for them."**
Many experts also disagree on the mortality rate of Covid-19. While the WHO gives estimates as high as 3.4%, **renowned epidemiologists such as John Ioannidis consider the risk is probably much lower, perhaps 0.125%, for which there are no reasons to take such draconian measures.**

France

In France, too, declarations of the Covid-19 pandemic seemed to have **a flair for strategic time and place.**

When Minister of Health **Agnes Buzyn** suddenly left office to replace a candidate who was running for mayor of Paris (he had to step down after a sex scandal), the coronavirus **crisis** seemed to be reasonably **manageable**. But the Covid-19 threat arose again at an opportune time — to ban large protests against a highly unpopular law that slashed pensions and on the eve of local March elections. After the first round of voting, a complete lockdown was announced. The former health minister, who wasn't elected mayor, expressed her regret for leaving office during the coronavirus crisis, saying that she knew from the start that the epidemic would escalate and soon turn into a major catastrophe…

But a disaster in France is easy to predict, as the situation is very similar to Italy. **1,300** public hospital doctors have been on administrative **strike** for almost a year. They refused to share the responsibility and decisions of a state that no longer provides minimal funds to run public health services. In the last two decades, the available number of beds has been **reduced** by 100,000 and the remaining facilities are largely **understaffed**.

Patients who died after waiting endless hours in the emergency room were already frequently reported by the media **long before** the coronavirus epidemic.

So the former health minister, who had received fierce

criticism for her inability to solve this lingering hospital crisis, **knew perfectly well that the coronavirus situation would further exacerbate the problem.** Recently, when President Macron visited doctors fighting the epidemic to show his support, medical staff took the opportunity to express their anger towards his **disastrous** health policies in front of the camera.

… [health authorities] replied that there was **not enough scientific evidence to prove efficacy and warned against potential side effects** of the [Chloroquine or Plaquenil], preferring to focus their efforts to find **new** molecules and develop a **new** vaccine, with France's **Sanofi Pasteur** included in the coronavirus vaccine competition.

The **silent war** in the treatment against Covid-19.
Finally, the Coronavirus epidemic **reveals the huge discrepancy between** the WHO health strategies and the reality for scientists and doctors who put patients' lives first.

The current **power** struggle in France about coronavirus strategies between health officials and the country's leading expert is truly eye opening. Professor **Didier Raoult,** who is one of the **world's top 5** scientists on communicable diseases and leads the high tech research center on infectious diseases, IHU – mediterranée

Marseilles, argued that the approach of **mass quarantine is both inefficient and outdated**.
Early on, Dr. Raoult suggested the use of hydroxychloroquine (**Chloroquine** or Plaquenil), a well-known, simple, and inexpensive drug. By mid-February, clinical trials at **his institute and** in China already confirmed that the drug could reduce the **viral** load and bring spectacular improvement. The Chinese scientists published their first trials on more than 100 patients and announced that the **Chinese** National Health Commission would recommend Chloroquine in their new guidelines to treat Covid-19. Chloroquine has sever side effects.

…last October, the French minister of health suddenly decided to put this long used over-the-counter drug on the list of "**controlled** substances" and make it a prescription drug.
As a member of a similar French committee, Dr. Raoult immediately shared the great news with health authorities. But they replied that there was **not enough scientific evidence to prove efficacy and warned against potential side effects of the drug**.

But Dr. Raoult and 600 members of his institute continued their work and confirmed similar results in a trial of 24 patients that was published March 3, 2020.
Dr. Raoult has recorded daily videos to share his research and knowledge, sometimes reaching half a million views

in a couple of days. Hospitals and general practitioners started to treat their patients with the drug **until it quickly went out of stock.**

In fact, for an **unknown** reason, last October, the French minister of health suddenly decided to put this long used over-the-counter drug on the list of "controlled substances" and make it a prescription drug.

While the WHO has repeatedly **praised** China and South Korea, for their "efficient response" using **draconian** quarantine measures, there has been **no** mention of the fact that those countries are using **Chloroquine.**

Now, a month later, under the growing pressure of doctors and the media, the government has finally decided to "consider more trials" of this protocol, and Sanofi Pasteur has announced that it will offer enough doses to potentially treat 300,000 patients.

Although Chloroquine was cited **second** on the WHO's original list of drugs to be evaluated for coronavirus treatment as a drug on its list of "essential medicines," the WHO has **not yet released any information about it and has not even mentioned the four clinical trials that received official European Union approval.**

Interestingly, on February 26, the United Kingdom put Chloroquine on its list of drugs that can **no longer be exported outside the country.** In the United States, a **white paper**, published on March 13 by researchers from the National Academy of Science and Stanford Medical School, **proposes** that "the United States of America and other countries should immediately authorize and indemnify medical doctors for prescribing chloroquine to treat COVID-19."

It looks as if the WHO and our Western governments have decided to **keep fueling the panic and raising the alert level, pushing the "Global Health Security Threat" narrative to the hilt.**

How much longer with this global lockdown last? Officials say "until a new vaccine has been developed," which will probably be in fast track mode by a well-known philanthropist after most courts in the world have ruled that **mandatory** vaccination does **not** violate human rights.

Or perhaps **until** the economy has completely **crashed** and can be **rebuilt** on a "healthy basis"? Here is a clue: the European Central Bank has launched a "Pandemic Emergency Purchase Program" that will last **until** "the coronavirus Covid-19 crisis phase is over, but in any case not before the end of the year"!

Gualtiero Ricciardi , known as Walter Ricciardi (born 1959), is an Italian doctor and **actor** .
As a doctor and university professor, he carries out his professional, didactic and scientific activity in the field of **Hygiene** and Public Health Medicine. Full Professor of Hygiene and Preventive Medicine, former Vice Dean of the Faculty of Medicine of the **Catholic** University of the Sacred Heart since 2012, he has held the position of Director of the Public Health Department of the Gemelli Polyclinic of **Rome** and **President** of the III Section of the Council Superior of Health . He was also president of the Italian Medical Manager Society (SIMM). He is founder and director of the Italian Observatory on Health in the Italian Regions since 2002. He was the first non-English editor of the Oxford Handbook of Public Health Practice , published by Oxford University Press , and the **first non**-American member of the US National Board of Medical Examiners; from 2010 to 2014 he was president of the European Association of Public Health, the association of **all public health companies** in the countries of the European Region of the **World Health Organization.** Active within the general political-economic guidelines of **Italia Futura** , for which he worked first as a **founding** partner and then as head of the Health and Health Department. He is a director of the Director of the European Region of the **World Health Organization** and Member of the Panel of Experts of the

European Commission for **investments** in healthcare in the European Union. In **2013-2014,** on commission of the **United Nations,** he coordinated the first benchmarking survey on the professional risks of the staff of the **World Food Program** , the UNHCR , the **World Bank** and the **International Monetary Fund.**

In September 2014, **Ricciardi**, and his colleagues **Charles Guest, Ichiro Kawachi** and **Iain Lang**, received the **British Medical Association award** as authors of the best Public Health book of the year, the Oxford Handbook of Public Health Practice. In February 2015, in **Calcutta** , he was elected representative of Europe in the Governing Council of the **World** Federation of Public Health Association (WFPHA), the World Federation of Public Health Societies. In July 2014, the ministers of health and economy of the **Renzi** government appointed him commissioner of the Istituto Superiore di Sanità - ISS, **the most important Italian health research institute.**
In September 2015, by decree of the President of the Council of Ministers, he was appointed **president** of the Higher Institute of Health , a position from which he announced his **resignation** in January **2019**, in controversy with the alleged lack of collaboration established in his opinion by the Conte I government and with the positions taken by important members of the executive on health issues and, in particular, on **vaccines** , waste-to-energy plants , the relationship between

immigration and the **spread** of diseases, defined by him as unscientific and anti-scientific. In 2014 he received the Krogh Award from the Danish Embassy in Rome and in 2015 the Takamine Award from the Japanese Embassy. Also in 2015, he was awarded the Edithe J. Levit Distinguished Service Award for services provided as a Member of the Board of Directors of the National Board of Medical Examiners of the United States of America. On 2 June 2017 the President of the Italian Republic Sergio Mattarella , on the proposal of the Prime Minister Gentiloni, appointed him **Commander** of the Republic in consideration of particular scientific and health merits. In November 2017, the Gentiloni government appointed him to **represent** Italy on the **Executive Board of the World Health Organization** for the three-year period 2017-2020.

His **acting** career began in the sixties, as a **child**, in the television series I bambini di Padre Tobia to continue in numerous television dramas (Love drama , A hero of our time , Our mother , The arrow in the side) and **continues** for all the eighties with **leading** roles alongside **actors** such as Giuliana De Sio , Alida Valli , Michele Placido , Stefania Sandrelli and Maria Schneider . Among his most interesting roles are those in the film I am mine (1978) by Sofia Scandurra , L'ultimo guappo (1978), Il mammasantissima and Napoli ... the camorra challenges

and the city answers (1979) **all** directed by Alfonso Brescia and played by Mario Merola .

Filmography
The Last Guappo (1978)
I am mine (1978)
The mammasantissima (1979)
Naples ... the Camorra challenges and the city responds (1979)
Stachel im Fleisch (1981)
Occhei, occhei (1983)
The difficult journey (1986)

Commendatore Ordine al Merito della Repubblica Italiana - nastrino per uniforme ordinaria… **Commander of the Order of Merit of the Italian Republic** - Rome , 17 July 2017

https://ec.europa.eu/health/expert_panel/sites/expertpanel/files/docs/body/ricciardi_en.pdf

A vaccine war.
In 2020, vaccines could weigh even more heavily in US elections. In fact, one could almost say that a vaccine war is going on across the US. After California, states like New Jersey, Maine, Connecticut, Virginia, Hawaii, Colorado **and many others are trying to adopt harsher vaccine laws.** But vaccine **freedom** advocates are getting

more organized, too, putting pressure on elected officials and candidates and even introducing their own legislation. For example, after the New Jersey legislature twice failed to pass a repeal of the religious exemption, even though Speaker Steven Sweeney vowed to "go to war" to get it passed, legislators proposed several vaccine safety bills. The Maryland legislature **refused** to allow pharmacists to administer vaccines, and in South Dakota, the legislature considered, although rejected, a bill that would have completely **prohibited** all medical mandates of any kind. **Europe** too is undergoing a similar **wave** of coercive legislation and pushback. In **Germany**, compulsory measles vaccination has just come into force in early March, even though the country has one of the highest coverage rates — 97% one dose, 93% two doses — and very few cases of illness or death. This vote comes two years after Chancellor Angela Merkel announced that there would be **no** mandatory vaccinations in Germany, as informed consent had "solid historical reasons." Everywhere in Europe — in Great Britain, Austria, Belgium, Romania, Slovenia, from Ukraine to Spain — **mandatory** vaccination bills are being introduced. Sadly, informed consent and the Nuremberg Code may now exist **only** in the museum of democratic values. The new German law is **particularly restrictive**. There is **no** option for home schooling, and the measles vaccine obligation applies to adults working in the health and education sectors as well. **But German citizens may be**

ready to fight back. Families and doctors are fighting the mandates in courts, and protests were planned all over the country for March 21, including a major event in Munich with Robert F. **Kennedy**, Jr. and activists from all over Europe – **until** the coronavirus pandemic intervened. Everywhere in Europe — in Great Britain, Austria, Belgium, Romania, Slovenia, from Ukraine to Spain — **mandatory** vaccination bills are being introduced. Faced with the **violation** of human rights that their Constitutions guarantee, people have filed complaints with the European Court of Human Rights. The Court, whose jurisdiction covers **49** countries throughout Europe and Eurasia, will hear cases on mandatory vaccination on **April** 30, 2020 arising from the Czech Republic.

It is undeniable that the coronavirus epidemic has come on the scene at a crucial moment, when people everywhere are in revolt against the power of international financial institutions **and** multinational pharmaceutical corporations, whose stranglehold on governments is **no longer** hidden. Many scandals have shaken confidence. The bankruptcy of an aberrant economic system is accelerating, ands attempts to start a **third world war** are multiplying. It is certain that many are seeking to have Covid-19 **serve** the political interests of a global governance project.

After having refused all outside help in the management of the pandemic, **Iran** made a complete about-face by **inviting** the WHO to its rescue.

Iran called again for lifting the ban and asked the International Monetary Fund for a $5 billion loan to fight the outbreak.

Cyrus Poonawalla & Bill Gates

GODFATHER OF VACCINATION

https://www.corvelva.it/en/approfondimenti/video/il-padrino-dei-vaccini-la-deposizione-di-stanley-plotkin.html

The curious case of the "vaccine hero" and the single anti-measles .. **Serum Institute of India, owned by the Poonawalla Group** and look who is the first "Hero of the Vaccines" in the world? The Dr Cyrus Poonawalla (who according to wikipedia reports is the **7th richest man in India, one of the 150 richest men in the world** according to Forbes). Just him, founder of the Serum Institute of India and president of the Poonawalla Group,

was awarded in December 2018, first in the world, of the "Vaccine Hero Award" by **Gavi**, the Vaccine Alliance.
https://www.corvelva.it/en/approfondimenti/sistema-sanita/case-farmaceutiche/il-curioso-caso-del-vaccine-hero-e-dell-antimorbillo-singolo.html

https://www.facebook.com/BillGates/photos/i-was-really-honored-to-be-welcomed-by-dr-cyrus-poonawalla-and-his-son-adar-poon/10150906643846961/

Serum Institute of India.
is a manufacturer of immunobiological drugs including vaccines in India. It was **founded** by Cyrus Poonawalla in 1966. The company is **the world's largest vaccine producer by number of doses produced.** It is currently developing an intra-nasal swine flu vaccine. In 2016, with support from US-based Mass Biologics of the University of Massachusetts Medical School, it invented a fast-acting **anti-rabies** agent, Rabies Human Monoclonal Antibody (RMAb), also known as Rabishield.

Cyrus S. Poonawalla (born 1941) is an Indian businessman, the chairman of Poonawalla **Group**, which **includes** Serum Institute of India, the Indian biotech company that manufactures paediatric vaccines. As per Forbes March 2018 rankings, Poonawalla's **net worth is Rs 73,000 crore** and is ranked the 7th richest person in India and the **170th** richest person in the world. He was awarded the Padma Shri for his contribution to the field of

medicine, by the Government of India in 2005. He also received the “Lifetime Achievement Award” by the then Prime Minister Manmohan **Singh** in 2005 . 2019 Poonawalla was awarded an Honorary Doctorate of Science by the University of **Oxford**.
In May 2019 it was reported that Poonawalla in partnership with Naum Koen have proposed supplying **Ukraine** with 100 thousand doses of measles vaccine for **free** vaccination.

http://www.gospanews.net/en/2020/03/07/pandemic-bioweapon-gsk-golden-vaccines-ring-with-bill-gates-pentagon-and-zionists-blackrock/

Walter Ricciardi, a member of the World Health Organization's executive council and Italian health ministry consultant on the coronavirus, provided a modest timeline last week. **Ricciardi suggested life could return to "normal" this summer. Ricciardi compared the coronavirus pandemic to** the SARS outbreak almost two decades ago, which he said **ended** in May or June. "I have the impression that, if we are lucky and all work together, we should get through to the **summer**," he said. "That's when we should be able to **return** to normal life."
https://www.usatoday.com/story/news/health/2020/03/15/coronavirus-crisis-end-summer-experts-odds-what-we-dont-know-epic/5053876002/

Italy: "ICU wards are overflowing"
by Jon Rappoport March 13, 2020

Some people, even if they have fallen over a cliff, would, on the way down, shout: "The virus must be dangerous! What else could it be?" **"People are dying! It's got to be the virus!" How about this?** "The ICU hospital wards in Italy are overflowing. It's the virus."

Step back and think. Think it through. Watching a recent interview with an Italian public health official, I had the impression that perhaps **several** thousand new ICU patients were burdening the hospital system in the northern part of the country. **Several thousand out of a national population of 60 million.**

Here's the **trick**. Before the announcement of the coronavirus epidemic, people who showed up at those hospitals, with flu, flu-like symptoms, lung infections, pneumonia would be placed in the general wards and treated, or even sent home with drugs. But now they would, many of them, be called "presumptive cases" of coronavirus, without any tests at all, **or after tests which don't work** (see my prior articles on why the diagnostic tests are **useless** and **deceptive**). By labeling these patients "contagious coronavirus cases," the

hospital doctors are forced to send them to the ICU, to "protect others from the infection." Thus, these ICUs **are crowded and overflowing.** The press publishes pictures of the ICUs and the **hysteria** factor bubbles up a few degrees hotter. The press interviews a hospital doctor, and he says, "We're starting to see a few more children with the virus." The public reaction? "Incredible! Now even healthy children are getting sick!" I have breaking news. Children do get sick. Like adults, they develop flu-like symptoms. And as with adults, they can now be diagnosed as "presumptive coronavirus cases."

"But what about people dying in Italy?" As in other countries, people in Italy do die. They always have. Especially old people, **who have all sorts of long-term health problems.** Labeling them with "coronavirus" at the last minute **doesn't** explain the cause of death.

"Healthy people in Italy are dying." Two points here. **First**, sometimes these healthy people **aren't** really healthy at all. And **second**, if you were healthy, and you were suddenly diagnosed, for no reason, with a virus you believed was dangerous and even deadly, and then you were isolated in an ICU ward, allowed no visitors, perhaps even put on a ventilator, and then treated with **highly toxic** antiviral drugs, do you think there is a chance you would die? The whole aim of stage magic is, as we all know, deflection of attention. The audience is

guided to look HERE, while the trick is being executed THERE. Here, a woman is being sawed in half. There, she is escaping from the back of the box. In the "epidemic," HERE is where people are sick and dying and diagnosed. THERE is where a fictional reason is being cooked up to explain why. "But…but…Italy, Italy, Italy, people dying, virus, virus…I don't get it…" Yes you do. You're getting the message the public health officials want to shove into your mind. You're standing on a street corner watching a pro execute his shell game, and you're falling for it every time.

Italy .. Coronavirus: New explosive information
by Jon Rappoport March 19, 2020

A very brief update. Read this carefully. Many people who were diagnosed as "coronavirus cases" in Italy, and then died, were almost certainly put on antiviral drugs. As you'll see, below, **a significant percentage of these people had prior heart conditions or high blood pressure.** But **at least one of** the antiviral drugs, called **ribavirin**, carries this **very relevant** warning, from cardiosmart.org: "Ribavirin may **decrease** the number of red blood cells in your body. This is called anemia and it can be life-threatening in people who have heart disease or circulation problems." High blood pressure is a

circulatory problem. Understand? Get it? **Life threatening** .

So how many coronavirus patients have been killed by the administering of ribavirin? And with that, let's jump in…because there's more. Much more. For those people who have any belief in the coronavirus… Here's the **basic** situation: the Italian health agencies are reporting escalating COV deaths—big fear-story out front… But in the **background**, other Italian government researchers are combing through patient records, to take a much closer look…to see whether people are dying from the virus **or other more obvious causes.** Are people dying coincidentally with the virus, or BECAUSE OF the virus? Is the virus a **mere harmless** passenger in the body, or is it the driving force? The Italian results are astonishing, to understate it by a mile. Bloomberg News has the story: 3/18/2020, **"99 percent of those whose died from virus had other illness,** Italy says": "More than 99% [!] of Italy's coronavirus fatalities were people who suffered from **previous** medical conditions, according to a study by the country's national health authority." "The Rome-based institute has examined medical records of about 18% of the country's coronavirus fatalities [so far, because it's slow work], finding that just **three** victims [!!], or 0.8% of the total, had **no** previous pathology [disease]. Almost **half** of the victims suffered from at least **three** prior illnesses and about a fourth had either **one or two** previous conditions."

"More than 75% had **high blood pressure,** about 35% had **diabetes** and a **third suffered from heart disease."** "The average **age** of those who've died from the virus in Italy is 79.5 [!!!]. As of March 17, **17 people under 50 had died from the disease. All of Italy's victims under 40 have been males with serious existing medical conditions."** BANG. Average age of those who've died: 79.5. Are you kidding? **Lots of prior medical conditions, weakened immune systems, and what this emerging study isn't saying: all these people** had obviously been treated for those prior conditions with **toxic** medical drugs. Furthermore, once they'd been diagnosed with coronavirus, chances are many of them were put on **highly** toxic antiviral drugs. Thus delivering the final blow. Imagining the coronavirus was the **CAUSE of death would be a ridiculous fantasy.** But these people are counted as "coronavirus deaths" by the other Italian reporting agencies, who are jacking up the numbers. Does this remind you of any other reports I've been detailing? The **elderly** people with obvious prior diseases who died in **Australia**; and the **elderly** people who were diagnosed as coronavirus cases in the state of **Washington—all** living in a long-term-care nursing home? Getting the picture? **This death-numbers con - aside from covering up the real causes of death,** including MEDICAL - is the forward spear being used to justify **locking down** and **wrecking economies all over the world** right now, and that means attacking the people

in any way connected to those economies who have to work to make a living. There are **statistical vampires** at work, using the **elderly** and **sick** and **dying** to feed numbers to health agencies around the planet. Those agencies tap their press contacts, and horror reports emerge, and the **unsuspecting** public, in economic lockdowns, sit in front of the tube and watch these reports, and inhale the cooked-up fear. Turn your mind to the highest setting, because nothing is riding on this whole deal **except** the immediate future of humanity. And again, cardiosmart.org: **"Ribavirin may decrease the number of red blood cells in your body.** This is called anemia and it can be life-threatening in people who have heart disease or circulation problems."

Corona Bologna Italy: The Truth begins to leak out.
by Jon Rappoport March 17, 2020

The government of Italy, as everyone knows, has locked down the whole country of **60** million people. So how many Italians have died from COV? Even by the standards of the **useless** and misleading diagnostic tests? Ready? As far as the Italian Higher Institute of Health knows, at this point: **Maybe two. Maybe.** Try to wrap your mind around that. Good luck. Seems the president of the Italian Higher Institute has some smarts. He understands that people **who already have other serious**

health conditions, which have nothing to do with COV, can and do die from those **other** conditions, regardless of the fact that they've tested positive (on **useless tests**) for COV. He gets it. I predict a great future for him. If he keeps shooting his mouth off, he might find himself working as a weed puller in a forest. Or he might suddenly be **diagnosed with** the virus and find himself in isolation. Grit your teeth and plow through this piece from Rome, 13 March 2020, Agenzia Nova: "Coronavirus: ISS [Italian National Institute of Health]: in Italy there are **only two deaths ascertained so far due to** Covid-19" (Italian, English) "There may be **only two** people who died from coronavirus in Italy, who did not present other pathologies. This is what emerges from the medical records examined so far by the Higher Institute of Health, according to what was reported by the President of the Institute [Istituto Superiore di Sanità (ISS), Italian National Institute of Health], **Silvio Brusaferro,** during the press conference held today at the Civil Protection in Rome. 'Positive deceased patients have an average of over 80 years – **80.3** to be exact…The majority of these people are carriers of **chronic** diseases. **Only two** people were not presently carriers of [other non-COV] diseases', but **even in these two** cases, **the examination of the files is not concluded and therefore, causes of death different from Covid-19 could emerge.** The president of the ISS has specified that **'little more than a hundred medical records' have so far come from hospitals**

throughout Italy." "…At present, in fact, the authorities are **unable** to **distinguish** those who died from the virus, **from** those who, on the other hand, are communicated daily to the public, but who were mostly carriers of **other serious diseases** and who, therefore, would **not** have died from Covid-19. In response to a question from 'Agenzia Nova', in fact, Brusaferro was **unable** to indicate the exact number of coronavirus deaths. However, the professor clarified that, according to the data analyzed, the vast majority of the victims **'had serious** [non-COV] pathologies and in some cases the **onset** of an infection of the respiratory tract can lead more easily to death.' To clarify this point, and provide real data, **'as we** acquire the folders we will go further. However, the populations **most at risk are fragile, carriers of multiple diseases'."** Translation into non-medical language: the people dying in Italy have other very serious traditional diseases that have nothing to do with COV, and it's obvious they could have died, and probably **did** die, from those other diseases. Nevertheless, we're **locking down** the whole country. So, for those people straining to find a reason for the "devastation" overtaking Italy—it's karma for ancient Rome trying to conquer half the known world; it's the ghost of Martin Luther obtaining revenge against the Vatican; it's a bioweapon with the power to cut down millions of people overnight; it's a virus that came in with a small meteor and crashed outside Milan; it's Chinese revenge against Marco Polo for stealing the concept of

noodles— Take a break, relax, have a plate of pasta, turn on the TV, and because all the stadiums are empty, watch a rerun of a soccer match from 1979. PS: For those people who believe this head of the Italian Institute is lying with his facts and figures, stop and think it through. He's going to announce such devastating news that essentially **contradicts** everything the Italian government is doing with its lockdowns and quarantines of the whole country? It would be **as if** the director of the Centers for Disease Control announced, "There are a total of nine deaths in the US we think might have been caused by COV, and even there we're **not** sure, because you see, these nine were elderly people who could barely get out of bed long before **COV** emerged. These nine had extremely serious lung disease **NOT CAUSED,** I repeat, **NOT CAUSED** by **COV**…but anyway, don't go outside, work from home, don't touch another human being, watch our website for bargain deals on toilet paper, and oh yes, don't forget to get your regular flu shot if you can slip into a hazmat suit and drive at breakneck speed to your nearest pharmacy, where injection clerks are waiting…"

Rockefeller Foundation Predicted the Coronavirus in 2010: The Good, the Bad and the Very Ugly
https://www.youtube.com/watch?time_continue=5&v=T8F6UN6eruU

Mao Was a Yale Man – Rothschilds Create People's Republic of China / Mao was a Yale Man – A Yali with Skull and Bones.
https://lipstick-and-war-crimes.org/mao-yale-man-rothschilds-create-peoples-republic-china/

DNA extraction **prior** to solution-phase**.**
PCR does **not** permit cellular localization of the amplified product. Several reports have described techniques whereby the **extreme sensitivity** of PCR is combined with the cell-localizing ability of in situ hybridization. **To enhance both the sensitivity and the specificity** of in situ PCR and PCR in situ hybridization, we developed a manual hot start modification, where an **essential reagent is withheld until the reaction temperature reaches at least 55**~ (8'9) This advance has obviated the need for the multiple primer pairs or tailed primers described by other investigators. (s'6) Retention of the amplified product at its site of origin is **essential** for successful in situ detection of PCR-amplified DNA and cDNA. The impetus for using **multiple** primer pairs and tailed primers was that they **dictated** the synthesis of overlapping amplified fragments. (s'6) It was **theorized** that the resultant large (>1000 bp) product was detected in situ because it resisted diffusion from its nuclear site of origin, whereas the smaller (450 bp) fragments were membrane-permeant. Two observations have **suggested** that under certain conditions there is minimal migration of

amplified product from its site of origin irrespective of size. First, amplified HIV-l-specific DNA sequences as small as 115 bp localized in situ to the nucleus in lymphocytes from AIDS patients and in infected cell lines. (9'11) In addition, amplified cDNA from either RNA **viruses** or **human mRNAs** showed distinct subnuclear and cytoplasmic localization. (12) However, we have noted that certain **fixatives**, such as **ethanol** and **acetone**, which support **standard** PCR (~3) appear to **hinder** successful in situ PCR. It is **unclear** whether this observation **reflects** loss of amplified product out of the cell **or** inhibition of PCR in the cell. The purpose of the present work was to define better the conditions that enhance the in situ localization of PCR-amplified DNA. These analyses documented the importance of various fixatives, fixation time, and protease digestion on retention of the amplified product as well as on nontarget DNA synthesis. Advantageous concentrations of various amplification **reagents** were **determined??**, as was the utility of a **single**-stranded DNA binding protein (SSB) in enhancing the **convenience** and the **sensitivity** of the technique when the **manual** hot start technique was **not** used. These results in conjunction with prior work on a wide variety of primer pairs (7'9'1L12) suggest that the conditions of fixation and protease digestion described in this work lend themselves to a generalized protocol for enhancing the specificity and sensitivity of the PCR in situ assay.

Cells and Targets Studied

Peripheral blood monocytes (PBMs) 2:305-312

9 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/93 $3.00 PCR Methods and Applications 305 Downloaded from genome.cshlp.org on April 9, 2020 - Published by Cold Spring Harbor Laboratory Press taken from a healthy volunteer were **isolated??** from a Ficoll gradient (Histopaque 1077, Sigma Diagnostics, St. Louis, MO).

About 5000 **cells??** were placed on silane coated glass slides (ONCOR, Gaithersburg, MD) using a **cytospin centrifuge** (900 rpm for 2 min); the cells were then fixed in **acetone**, 95% **ethanol**, and 10% neutral buffered **formalin** (10% formalin in 0.1 M **sodium phosphate** buffer at pH 7.0), or **Bouin's** solution (75 parts saturated **picric acid** 25 parts 40% **formaldehyde** in water, and 5 parts **glacial acetic acid**; at pH 1.6).
By placing two cytospins on a slide, direct comparisons of paired experimental conditions were done. **Protease** digestion, when used, was done after fixation. **Pepsin** (Life Technologies, Gaithersburg, MD) digestion was done in 0.01 N HCI at **room** temperature; the concentration was 2 mg/ml and the time 12 min unless otherwise indicated. **Proteinase** K (ONCOR) digestion at 1 mg/ml for 12 min was done in water. Also studied was the human cervical cancer cell line SiHa (ATCC HTB 35), which contains one integrated copy of human

papillomavirus (HPV) 16 DNA per cell. (~4) The SiHa cells were fixed for 15 hr in buffered **formalin** and embedded in **paraffin**; multiple 4 i~m sections were placed on glass slides. The PCR **primers** were specific for a region of the oncogene bcl-2, which should be detectable in all PBMs, the type-specific E6 region of HPV 16, and the nucleocapsid region of the measles virus. Also employed was a Burkitt's lymphoma cell line (ATCC VR-603) which contains EBV DNA. These were mixed with oral squamous cells to demonstrate the specificity of the in situ PCR signal when using EBV-specific primers. Primer sequences are listed in Table 1. In Situ PCR In situ PCR refers to the technique whereby (digoxigenin) **labeled** nucleotide is directly incorporated into the amplified product in fixed cells. Our technique, which includes the **manual hot start** modification, has been described previously. (8-12) Briefly, 7.5 p.1 of the amplifying solution, containing buffer (GeneAmp kit, Perkin-Elmer **Cetus** Corporation, Norwalk, CT), 4.5 mM MgC12, and 1 ~M **each of primers,** 200 IJ.M dNTPs and 10 p.M digoxigenin -11-dUTP (Boehringer Mannheim, Indianapolis, IN), was placed over the fixed cells on TABLE 1 Sequences of the Primers Used in This Study Target a'h Sequence Product size/reference (bp) HPV 16 primer 1 nt 110 primer 2 nt 559 bcl-2 primer 1 nt 2779 primer 2 nt 3283 Measles primer 1 nt 115 primer 2 nt 549
5'-CAGGACCCACAGGAGCGACC S'-TTACAGCTGGGTTFCTCTAC 5'-CATTTCCACGTCAACAGAATTG 5'-AGCACAGGATTGGATATTCCAT S'-

GTGTAATAATATCATGGTTA S '-CTCTCCAATCTAAATTCACC 449 (7) 504 c 434 (23) aThe EBV-specific **primers** used in this study were published by Saito et al. (24) bnt = nucleotide position of primer S' end in the GenBank sequence. CKindly supplied by Dr. Ernest Kawasaki. the glass slide and covered with a plastic coverslip, which was then anchored to the slide with nail polish. The slide was placed in an aluminum foil "boat," which was placed directly on the sample block of a **thermal** cycler (Perkin Elmer Corporation). Two and one-half microliters of the DNA **amplifying solution** containing 2.0 units of **Taq DNA polymerase** (AmpliTaq DNA polymerase, **Perkin Elmer Corporation**) was **withheld** from the initial amplifying solution. When the **temperature of the heating block reached 65~ the coverslip was partially lifted and the enzyme added.** The slide was then overlaid with - 1 ml of **mineral oil preheated to 82~** After an **initial denaturing** step of **94~ for 3 min, 20 cycles were accomplished using the following protocol: annealing/extension at 55~ for 2 min and denaturation at 94~ for 1 rain. The mineral oil was removed with a 2 min xylene wash, and the xylene was then removed with a 2-rain 100% ethanol wash.**

Detection of **digoxigenin** incorporated into PCR product was done with **alkaline** phosphatase-conjugated antidigoxigenin-labeled **antibody** at a 1:50 dilution according to the **manufacturer's** protocol (Boehringer Mannheim). The alkaline phosphatase-based colorimetric

detection method used the **chromogen nitroblue tetrazolium** (NBT) which, in the presence of **5-bromo-4-chloro-3-indolylphosphate (BCIP), yields a purple-blue precipitate as the marker** of a positive cell. The **counterstain, nuclear fast red** (ONCOR), stains nuclei pale **pink** and does **not** stain cytoplasm.

PCR
In **Situ** and **Southern** Blot Hybridizations
In PCR in situ hybridization, the **unlabeled amplified** DNA is **detected** using a **labeled** probe.
The **two methods** use the **same** amplification, detection, and staining procedures **except** that digoxigenin dUTP is **omitted** from the PCR **reagents** when a labeled probe is to be used. Our high-stringency in situ hybridization protocol has been published previously.(1 s) A digoxigenin-labeled genomic HPV 16 probe made by the random primer method (Genius kit, Boehringer Mannheim) was used for detection of amplified HPV 16 DNA. **Southern** blot hybridization **analysis** of amplified bcl-2- specific sequences in the amplifying solution and cellular extract was done with a 32p-labeled oligonucleotide internal fragment that spanned nucleotides 2977-2992, using a previously reported **protocol**. (16) Chemical Hot Start The SSB from E. coli (U.S. Biochemicals) was used in PCR in situ hybridization analysis of HPV 16 DNA in SiHa cells. In these experiments, **all reagents, including** the Taq DNA

polymerase and the SSB, were **added** to each sample in a 10 ~l volume **before** elevating the **temperature** of the block of the **thermal cycler.** The molar equivalent **ratio** of SSB to total primer (2 I~M) ranged from 50:1 to 1 : 210, **calculated** from the protein concentration of 2.3 i~g/l~l. A molecular mass of 75.6 kD was **calculated** for the tetramer, as was reported by the SSB **supplier** and the ability of one SSB tetramer **to bind two** oligonucleotides under the salt conditions (4.5 mM MgC12 and 45 mM KCl) used in this study. (17) (This study examined NaCl; we are **assuming** the calculations to be equivalent with KC1.) The experiments tested the effect ((306 PCR Methods and Applications Downloaded from genome.cshlp.org on April 9, 2020 - Published by Cold Spring Harbor Laboratory Press TABLE 2 The Effect of Fixation Chemistry and Duration on the Detection of Amplified bcl-2 DNA in PBMs with No Protease Digestion Detection of bcl-2-amplified DNA (% positive cells) (fixative) Fixation time **formalin acetone** 95% ETOH Bouin's 5 min 5 2 14 0 15 hr 0 15 31 0 39 hr ND 0 9 ND (ND) **Not done.))** of SSB on the amount of HBV 16-specific DNA synthesis, as **only** HBV 16 target sequences would be detected.

RESULTS

Every human PBM will contain **two** copies of the bcl-2 sequence that is amplifiable by the primers used in this study (Table 1). To determine the importance of fixation

chemistry and duration, in situ PCR was performed on PBMs fixed for 5 min, 15 hr, or 39 hr in **acetone**, 95% ethanol, buffered formalin, or Bouin's solution. Each experiment was **repeated at least once,** and the **tabulated values are the mean scores;** the range of variation between replicate experiments was from 0% to 9%, and the **highest** standard deviation was 9. The results are compiled in Table 2; Figure 1B shows a representative photomicrographs. **Under no condition did all PBMs have detectable amplified bcl-2 DNA.** The **low** and variable detection efficiencies suggested that the fixation conditions **may limit** cell permeation **by a key reagent.** To test this **hypothesis**, the cells were digested with 2 mg/ml of **pepsin** for 12 rain prior to , in situ PCR. The results for in situ PCR **after protease digestion** are listed in Table 3. The percentage of positive cells **after** protease digestion **increased** to 100% after a 15 hr fixation with **formalin** (Fig. 1C) but **decreased** to 0% for the **acetone**- and **ethanol**-fixed cells. Cells fixed in **Bouin's** solution did **not** demonstrate a positive signal under any reaction conditions. Because cell morphology was poorly preserved **after** protease digestion and acetone and 95% ethanol fixation, or **after** a 5-min formalin fixation, in situ PCR was performed after various fixation times with the **pepsin** concentration decreased to 20 ~g/ml and the time of digestion varied from 1 to 12 min. As shown in Table 3, **no** increase in the percentage of positive cells with **decreased** protease digestion time and concentration was

evident for the acetone- or ethanol-fixed cells. However, after a 5-rain fixation in buffered **formalin** the positive cells increased from 0% with a 12-rain digestion with 2 mg/ml of **pepsin** to 35% with a 3-min digestion with 20 ~g/ml **pepsin**. Note the subsequent decrease to 0% for the latter if the protease time was increased to S min, indicating a **narrow** optimal threshold. **Alternative explanations for the low** detection efficiencies in most experiments were **that** the DNA was amplified and migrated out of the cell **or** that the fixation conditions blocked in situ bcl-2 amplification. To **test** these **hypotheses**, the amplifying solution was retrieved, its DNA separated on an agarose gel, and DNA sequences homologous to the internal fragment of the bcl-2 gene were analyzed using a 32p-labeled probe and Southern hybridization. These blots showed (Fig. 1A) that there was **marked** specific amplification evident in the solution from the cells fixed for either 5 min or 15 hr in acetone or ethanol with **no** protease digestion step. **No** detectable extracellular signal was evident in the solution from the cells fixed in buffered **formalin** for either 5 rain without digestion (conditions giving 5% positive cells) or 15 hr with protease digestion (conditions giving 100% positive cells). To demonstrate that the signal seen in the cells fixed for 15 hr in buffered formalin and then digested was target specific, the digoxigenin-tagged DNA was **extracted** from the cells after in situ PCR as described previously,(11,1 s) electrophoresed on an agarose gel,

blotted onto a **nylon** membrane, and analyzed for both immobilized digoxigenin and the ability to hybridize to a 32P-labeled probe to the FIGURE 1 Effect of fixation conditions on the loss of amplified bcl-2 DNA from fixed PBMs. (A) Southern hybridization analysis of the **supernatant** amplifying **solution** recovered from selected in situ PCR reactions described in Tables 2 and 3. Lanes a and d report on acetone fixation for 5 min and 15 hr, respectively, without protease treatment (Table 2). Lanes c and freport on 95% ethanol fixation for 5 min and 15 hr, respectively, without protease treatment (Table 2). Lanes b and e report on formalin fixation for 5 min without proteolysis (Table 2) and 15 hr with proteolysis (Table 3), respectively. The arrowheads mark the position of the 500-bp band of HindlII digest of kDNA. Note that the amplifying solution from the acetone- and ethanol-fixed cells, but **not** the formalin-fixed cells, contained the amplified bcl-2 product. (B, C) Representative microscopic fields for the histochemical analyses corresponding to lanes b (5 min formalin, no protease, 5% positive cells) and e (15 hr formalin, with protease, 100% positive cells), respectively. Pale images (open arrow) indicate light-red counterstaining in the absence of the dark-blue phosphatase-linked staining of digoxigenin-tagged localized DNA (solid arrow).

TABLE 3 The Effect of Fixation Chemistry and Duration on the Detection of Amplified bcl-2 DNA in PBMs with Pepsin Digestion Fixation time/pepsin conditions

Detection of bcl-2-**amplified** DNA (% positive cells) a (fixative) formalin acetone 95% ETOH Bouin's 5 min/2 mg/ml, 12 min 0 0 0 0 5 min/20 ~g/ml, 1 min 1 0 0 -- 5 min/20 ~.g/ml, 2 rain 35 0 0 -- 5 min/20 ~.g/ml, 5 min 0 0 0 -- 15 hr/2 mg/ml, 12 min 100 0 0 0 aCompare these data to the first two rows of Table 2. bcl-2 intervening sequence. A digoxigenin-labeled band, which hybridized to the probe, was evident at the **expected??** size of 504 bp in the cell extract but **not** the **amplifying solution** (Fig. 2). Alternatively, a 504-bp digoxigenin band that hybridized to the probe was detected in the amplifying solution and not the cell extract for cells fixed for 5 rain in formalin and proteased for 12 min at 2 mg/ml (conditions giving 0% positive cells; Table 3). To determine whether the potential **depurinating effect** of the low pH (2.0) of the pepsin digestion may have contributed to the results noted with the different fixatives and bcl-2 in PBMs, the experiments with ethanol and buffered formalin-fixed PBMs were **redone** with proteinase K digestion. No differences were observed in the rate of bcl-2-positire cells when compared with the results of pepsin digestion, including a detection rate of

100% for 15 hr **formalinfixed**, proteinase K-digested cells. When the PCR **cycle number was varied from 1 to 20 for** PBMs fixed **15 hr in formalin and digested with pepsin, staining** was **first** evident **after four cycles and was maximized after seven cycles.** The signal was present predominantly in a diffuse way in the nucleus, although at times, perinucleolar staining was evident. The most **advantageous** MgC12 concentration was determined by subjecting the 15-hr fixed/pepsin-digested PBMs to in situ PCR in 0, 1.5, 4.5, 6.0, and 9.0 mM MgC12 for 7 cycles and 20 **cycles** each. **No** signal was evident when the amplifying solution contained **no** MgCl2 or 9.0 mM MgCl 2. **A weak signal was evident only after 20 cycles** for 1.5 and 6.0 mM MgCl2, compared with the **intense** signal seen with 4.5 mM MgCl 2 (Fig. 3). A similar study of Taq DNA polymerase concentration showed that a signal was evident with 2.0 U/10 ~1 of amplifying solution but not with 0.4 or 0.2 U/10 pd. Because 2.0 U/10 ~l of Taq DNA polymerase is **10 times higher than required for standard** PCR, it was **reasoned???** that some of the enzyme might be sequestered on the glass slide. The **addition** of 1 mg/ml of bovine serum albumin (BSA), which could block enzyme adsorption, resulting in a strong signal even with 0.2 units of Taq DNA polymerase/10 p.l (Fig. 3). Previously, we found that the manual **hot** start modification described in Materials and Methods enhanced both the sensitivity and specificity of in situ

PCR. ~7~ SSB has been shown to have similar effects on standard PCR. (17'1s) In experiments to test the effects of an SSB on target-specific in situ amplification, SiHa cells were employed with primers specific for the one copy of HPV 16 present in each cell. PCR in situ hybridization was done using a digoxigeninlabeled HPV 16 probe where **all amplification reagents, including the Taq DNA polymerase** and SSB at various concentrations, were **added before increasing the temperature of** the thermal cycler. The results are depicted in Figures 4 and 5. An intense hybridization signal was evident only with a molar equivalent ratio (SSB : primer) of 1 : 21 (Figs. 4 and 5). Nonspecific DNA synthesis in in situ PCR is **presumed???** when a signal is evident with **nonsense** primers. The nonsense primers tested here were **measles-specific**, and the cells studied were PBMs from an **uninfected** individual. As **no** reverse transcriptase step was employed, **even if** measles RNA was present in cells, which **is highly doubtful, it should not be amplifiable.** Manual hot start in situ PCR was employed. The data are compiled in Table 4. Note that the rate for 15 hr-fixed cells digested for 12 min was **decreased** dramatically (77-0.02%) with the hot start modification. Interestingly, the **rate of positive cells was -80% if the digestion time was increased to 30 min for the cells fixed in formalin for 15 hr.** This rate was **not** reduced by **increasing** the annealing temperature to 65~ This **high nonspecific rate in overdigested cells was amplification dependent, as**

omission of the enzyme eliminated the staining. FIGURE 2 **Specificity** of in situ PCR amplification of bcl-2 sequences, bcl-2 target was **amplified by either** solution-phase hot start PCR (10 ng **purified** PCR product, nucleotide 2779-3283 bp) or in situ PCR on PBMs formalin-fixed for 15 hr and pepsin digested (2 mg/ml for 12 min); in each case, digoxigenin-dUTP was incorporated during 15ocycles using the same cycle parameters. After in situ PCR, **total cellular DNA was extracted by phenol/chloroform, then ethanol precipitated.** Agarose gel electrophoresis of solution-phase PCR product (lane a of each panel), DNA recovered from fixed cells (lane b of each panel), and the supernatant (lane c of each panel) was followed by **blotting** onto a nitrocellulose **filter**. The arrow marks the position of the 500-bp band of HindlII digest of k DNA. (A) Phosphatase-linked histochemical staining of digoxigenin; (B) the autoradiograph after hybridizing the blot to the 32P-labeled bcl-2-specific internal probe. **Amplified** bcl-2 DNA is detectable in each panel in the DNA **extracted** from the cells , **but not** in the amplifying solution.

FIGURE 3 Effect of MgCl 2 concentration of the detection of amplified bcl-2 DNA in PBMs.

At a MgC12 concentration of 4.5 mM all PBMs fixed for 15 hr in buffered formalin and digested had an **intense signal**??? using in situ PCR and bcl-2 primers (upper left). **No signal is evident in** PBMs analyzed on the same glass slide if no MgCl 2 is added (upper right). The Taq polymerase concentration is 2 units per reaction in each panel. A Taq polymerase concentration of 0.2 units per reaction gives a **greatly diminished signal** in PBMs fixed for 15 hr in buffered formalin and digested (lower right) compared with that evident with 2.0 units of enzyme (upper left). The **signal is enhanced if** 2 mg/ml of BSA is **added** in a parallel reaction (0.2 units of Taq) on the same glass slide (lower left). MgC12 concentration = 4.5 mM for lower right and lower left. The **Southern** blot presented in Figure 2 demonstrates the specificity of the in situ bcl-2-amplified signal in PBMs. To test the **specificity** of the in situ PCR technique using the manual hot start modification and another system, squamous cells from the oral cavity were admixed with the Epstein-Barr virus (EBV)-infected lymphocytes from the Burkitt's lymphoma cell line. Primers specific for EBV were employed. A signal in the squamous cells, which were in situ hybridization. In these experiments, **all reagents, including the Taq DNA polymerase** and the SSB were added to each sample in a 10-1~1 volume before elevating the temperature of the block of the thermal cycler. **No** hybridization signal is evident in SiHa cells that contain

one copy of HPV 16 per cell after in situ hybridization analysis with a digoxigenin-labeled HPV 16 probe **after** PCR if SSB is added at a **molar** equivalent ratio to primer of 1 : 210 (A); a signal is evident with the manual hot start modification (not shown here)/7~ A hybridization signal is evident **if SSB is added at a ratio of** 1 : 21 in a **parallel** experiment done on the same glass slide (B). … **negative**, **must represent nonspecific** DNA synthesis. The results are provided in Table 5. **Note the absence of any signal in the squamous cells and** the similar results with the different fixatives when compared with the data for the PBMs and the bcl-2-specific primers (Tables 2 and 3). A representative photograph is presented in Figure 6.

DISCUSSION

This study characterized **conditions that enhance in situ detection of** PCR-amplifled DNA. In this study only buffered formalin fixation for 15 hr with protease digestion allowed detection of amplified DNA in every cell that contained the target. The MgC12 concentration of 4.5 mM maximized signal in the cellular preparations for bcl-2 detection. The conditions of formalin fixation, protease digestion, and 4.5 mM MgC12 outlined here **appear** to be a generalized protocol for in situ PCR as one obtains the **strongest** signal under these conditions for **a variety of primers and** targets that ranged in size from 115 to 833 bp and included HPV 6, 11, 16, HIV-1, EBV, and cDNAs for measles, tumor necrosis factor, and

other human mRNAs/7-9'~1'12~ **The need for relatively high concentrations of Taq DNA polymerase likely reflects, at least in part,** sequestration of the enzyme on silanized glass, as shown by **signal enhancement at lower enzyme concentrations with the addition of BSA.** The ability to PCR-amplify sequences from tissues fixed in either ethanol or acetone is well documented**???**. Greer et al. ~13~ demonstrated that successful PCR was **more likely** for DNA extracted from tissues fixed in either acetone or ethanol **than** for DNA subjected to buffered formalin. However, the present study showed that amplification **occurred** in ethanol- and acetone-fixed cells, **but** PCR product was **primarily** detectable in the **amplifying solution**. Thus, **neither** ethanol nor acetone fixation will **prevent migration** of the amplified product out of the nucleus, **probably** because proteins and, perhaps, nucleic acids are **not** crosslinked. In **contrast**, prolonged formalin fixation was clearly able to create a physical **barrier** to prevent migration of PCR product. **Formalin** (i.e., formaldehyde) **extensively polymerizes proteins and can cross-link** nucleic acids (10-22~ which probably is the **essential** step for **limiting** PCR product diffusion.

1:210 1:62 1:21 1:7 1:2 50:1 3 o [,q 2 a:1 SSB: Primer (molar equivalent ratio)

Effect of SSB concentration on the detection of HPV 16 DNA by PCR in situ hybridization without the manual hot start modification. The presence and intensity of the hybridization signal evident with the addition of SSB at different molar equivalent ratios to the total primer concentration (2 tzM) is depicted. The signal intensity was visually graded by one of us (G.J.N.) from 0 (no signal) to 4 (intense) without knowledge of the SSB concentration. gration limitation is striking based on several observations. **First**, amplified cDNAs corresponding to human mRNAs localize to specific cytoplasmic and subnuclear compartments. r **Second**, amplified HPV 16 DNA is detectable in most **paraffin**-embedded SiHa cells even after the nucleus is sectioned; **presumably** (((TABLE 4 The Effect of Manual Hot Start on the in Situ PCR Detection Rate Using Nonsense (Measles-specific) Primers on PBMs Fixation time/ Percentage pepsin conditions positive cells 5 rain/no protease 0 5 min/20 I~g/ml, 2 rain ~ 0.02 15 hr/no protease 0 15 hr/2 mg/ml, 12 rain ~ 0.02 b 15 hr/2 mg/ml, 30 min 80 The numbers were based on -10,000-15,000 **cells???**. aThe detection rate was 0 **if** the annealing/ extension temperature during cycling was **increased** from 55~ to 65~ t'The detection rate for 15-hr **formalin**-fixed cells digested for 12 **min** with **pepsin without** the manual

hot start modification (**all** reagents added before temperature of block was elevated) was 77%.))) some **positive** cells **still** contained the one copy of HPV 16 after part of their **nuclei???** is **removed by** the microtome blade. (7'9) **Third**, no amplified product is detectable in the amplifying **solution** after in situ PCR with formalin-fixed, digested cells when all of the cells have intense **nuclear** staining. Formalin cross-linkage, which we **theorize** suppresses migration of the amplified product, **apparently** also can inhibit ((TABLE 5 Effect of Different **Fixatives** on the Detection of PCR-Amplified DNA Using EBV-Specific Primers in a Mixture of an EBV-**Positive** Cell Line (Burkitt's Lymphoma) and Viral-**negative** Squamous Cells Detection of amplified DNA (% positive cells) Burkitt's Squamous Fixative/time lymphoma cells Acetone/5 rain 9 0 Acetone/15 hr 16 0 95% Ethanol/5 rain 20 0 95% Ethanol/15 hr 22 0 Formalin]l 5 hr/ protease a 100 0 aProtease is pepsin at 2 mg/ml for 12 rain.)) entry of **at least one key** reagent, necessitating a protease digestion step. This effect is evident even after 5 min of fixation, as the detection rate under these conditions without proteolysis was -5% and **no** product was detectable?? in the amplifying solution. Protease digestion could **not** increase the detection rate to 100% for in situ PCR of cells fixed for 5 min in formalin; the detection of PCR product in the **amplifying** solution after **longer** pepsin digestion for cells fixed for 5 rain **suggested??** that too few cross-links had been formed to

create a robust migration barrier. It **appears** that protease digestion **may be** a necessary step , within situ PCR **when** cross-linking fixatives are used. **Both** solution phase and in situ PCR **suffer from several side reactions that compete with target-specific amplification.**

Nonspecific DNA synthesis can follow extension of **primers** annealed to **non**target sequences ("**mis**priming") and extension of primers onto **one another's** sequence to form primer **oligomers**; the **latter** process needs **no** additional DNA. (s'8~ We have shown previously that manual hot start PCR, a method whereby **at least one reagent is withheld until** the reaction temperature has reached at least 55~ at the initiation of cycling, **greatly reduces?? side reactions and concomitantly increases?? the yield of specific product** in situ. (8~ In the prior study, ~s) where PBMs were mixed with SiHa cells and primers **specific** for HPV 16 were employed, we demonstrated that with standard in situ PCR both the PBMs and SiHa cells incorporated labeled nucleotide. With the hot start modification, labeled nucleotide was incorporated **only** in the SiHa cells??. In an analogous experiment, PBMs were **mixed** with measles-infected HeLa cells, which can be **differentiated??** from the PBMs **by** their multiple nuclei. Using **reverse transcriptase** in situ PCR with direct incorporation and measles-**specific?** primers, it was shown that **only** the HeLa cells incorporated signal under the optimal conditions described in this study. ~12'21) **Equivalent**

results were obtained in this study using squarnous cells and lymphocytes infected by EBV and EBV-specific primers. These observations all highlight the **specificity??** of the PCR product synthesized inside cells. The specificity?? was **documented** by Southern blot hybridization with an internal probe of the PCR-amplified bcl-2 DNA 3 ! 0
((PCR Methods and Applications Downloaded from genome.cshlp.org on April 9, 2020 - Published by Cold Spring Harbor Laboratory Press FIGURE 6 The specificity of in situ PCR as demonstrated by cell-mixing experiments.)) An EBVpositive Burkitt's lymphoma cell line was **mixed** with viral-**negative** oral squamous cells, fixed for **15 hr in formalin,** and digested in pepsin (2 mg/ml) for 12 min. With the manual hot start maneuver and EBV-specific primers, all of the lymphocytes had a signal with direct incorporation of digoxigenin-labeled nucleotide into the PCR product (open arrows). None of the squamous cells, which are **easily** distinguished??? cytologically by their ample cytoplasm (solid arrows), had nuclei with a detectable signal. extracted from the PBMs aRer in situ PCR (Fig. 2). The present study shows that **although nonspecific incorporation can occur** in hot start in situ PCR, it is easily **limited** to a rate of -1/5000 cells as **compared** with a rate of > 50% **when this modification is omitted**. This **rate** was **dependent** on the annealing/ extension **temperature**, whereas the rate of target-**specific** amplification was **not** temperature

dependent **up to 65~** However, it is **unclear** why extended protease digestion should **increase non**specific DNA synthesis **so dramatically, even at the higher annealing temperatures.** We have noted a **similar high, non**specific incorporation rate for in situ PCR in formalin-fixed tissue sections **even after** 12 rain of proteolysis with **nonsense** measles primers (G.J. Nuovo, unpubl.). The **reasons** for these observations **will require further study.** The **enhanced sensitivity** that is the **hallmark** of hot start PCR was **evident without** the manual hot start maneuver **when** an optimal concentration of SSB was **added** to the amplifying solution. At a molar equivalent ratio (to total primers) of 1:21, the SSB may **inhibit** mispriming **without** binding so much primer , that **specific** amplification is **blocked**. The **complete blockage of specific** amplification at SSB concentrations **near and above** the primer concentration is the **expected** result of primer sequestration. The **failure** of specific amplification at **very low** SSB concentrations should occur **if** side reactions overwhelm the specific reaction. We documented this occurrence in PCR and PCR in situ hybridization when manual hot start is omitted. http://genome.cshlp.org/content/2/4/305.full.html#ref-list-1 https://genome.cshlp.org/content/2/4/305.full.pdf

- Denaturation = 96 degree C
- Annealing = 55 degree C
- Extention = 72 degree C

- Taq polymerase = enzyme from bacteria Thermes Aquaticus.
- exponential = 6 hours = 40 cycles = i trillion 99 billion .... copies ??? // SARS cov2 = RNA virus ... reverse transcriptase enzyme

Each PCR cycle **theoretically** doubles the amount of targeted sequence (amplicon) in the reaction. Ten cycles **theoretically** multiply the amplicon by a factor of about one thousand; 20 cycles, by a factor of more than a million in a matter of hours. Each cycle of PCR includes steps for **template denaturation**, primer **annealing** and primer **extension**. The initial step denatures the target DNA by heating it to 94°C or **higher** for 15 seconds to 2 minutes. In the denaturation process, the two intertwined strands of DNA separate from one another**???**, producing the necessary single-stranded DNA template for replication by the thermostable DNA polymerase. In the next step of a cycle, the temperature is **reduced** to approximately 40–60°C. At this temperature, the oligonucleotide primers can **form stable associations (anneal) with** the denatured target DNA and **serve as** primers for the DNA polymerase. This step lasts approximately 15–60 seconds. **Finally**, the synthesis of new DNA begins as the reaction temperature is **raised** to the optimum for the DNA polymerase. For **most** thermostable DNA polymerases, this temperature is in the

range of 70–74°C. The extension step lasts approximately 1–2 minutes.
The **next** cycle **begins** with a return to 94°C for denaturation. Each step of the cycle should be optimized for each template and primer pair combination. If the temperature during the annealing and extension steps are **similar?**, these two steps can be combined into a single step in which both primer annealing and extension take place. After 20–40 cycles, the amplified product may be analyzed for size, quantity, sequence, etc., or used in further experimental procedures.

RT-PCR
Thermostable DNA polymerases used for **basic** PCR **require** a **DNA** template, and as such, the technique is **limited** to the analysis of DNA samples. Yet numerous instances exist in which amplification of **RNA** would be preferred. To apply PCR to the study of RNA, the RNA sample must **first** be **converted** to cDNA to provide the necessary DNA template for the thermostable polymerase (Figure 1). This **process is called reverse transcription** (RT), hence the name RT-PCR. Reverse transcriptases (RTs) **are** , RNA-directed DNA polymerases that were **first** identified as **part** of the retroviral life cycle (Temin and Mizutani, 1970, Baltimore, 1970). RTs **catalyze** the synthesis of a DNA copy (cDNA) of the target RNA molecules using a reverse transcription **primer**, dNTPs, and Mg2+ or Mn2+ as a cofactor. Reverse transcriptases

have been **adapted** for use in a variety of in vitro applications including **real-time** and **endpoint** RT-PCR, **labeled**-cDNA probe generation and cDNA library construction. The ideal reverse transcriptase is robust (highly active under a variety of conditions) and converts all primed RNA within a sample to cDNA, regardless of its abundance, length or secondary structure. **The most** characterized RTs used for molecular biology **are the retroviral** RTs: **avian myeloblastosis virus** (AMV) and **Moloney murine leukemia virus** (M-MLV or MuLV)).

Genetic engineering and development of proprietary RT-enhancing buffers have led to the **commercial** availability of **new enzymes that offer superior performance over these naturally** occurring RTs. AMV and M-MLV reverse transcriptases are generally used to produce a DNA copy of the RNA template **using** either **random** primers, an **oligo**(dT) primer or **sequence-specific** primers. Some thermostable DNA polymerases (e.g., Tth DNA polymerase) **possess a reverse transcriptase activity**, which can be **activated by** using **manganese instead of magnesium as a cofactor** (Myers and Gelfand, 1991). After this **initial** reverse transcription step to produce the cDNA template, **basic** PCR is carried out to amplify the target sequence. The quality and purity of the RNA template is **crucial** to the success of RT-PCR. Total RNA or poly(A)+ RNA can be used as the **starting** template, but both must be **intact** and **free** of

contaminating genomic DNA. **Specific** capture of poly(A) + RNA will **enrich** a targeted message so that **less** of the reverse transcription reaction is needed for subsequent amplification. The efficiency of the **first**-strand synthesis reaction, which can be related to the RNA quality, also will **significantly** affect amplification results. GoScript™ Reverse Transcriptase is a formulation of M-MLV reverse transcriptase **and optimized buffers** designed for efficient and reproducible synthesis of **first**-strand cDNA **from** a full range of rare and abundant transcripts, even with **difficult** templates and in the presence of PCR **inhibitors**. GoScript is qualified for use in qPCR and is **compatible** with GoTaq® RT-qPCR Systems. GoScript is available in convenient mixes with either Oligo(dT) primers **or** random primers, as part of a complete kit, **and** as a **stand-alone enzyme.**

Hot-start PCR is a common technique to **reduce nonspecific** amplification due to assembly of amplification reactions at room temperature. At room temperature, PCR primers can anneal to template sequences that are not perfectly complementary. Since thermostable DNA polymerases have activity at these low temperatures (although in most cases the activity is less than 25%) the polymerase can **extend mis**annealed primers. This newly synthesized region then acts as a template for primer extension and synthesis of undesired amplification products. However, if the reaction is heated

to temperatures >60°C before polymerization begins, the stringency of primer annealing is increased, and synthesis of undesired PCR products is avoided or **reduced**. Hot-start PCR also can reduce the amount of primer-dimer synthesized by increasing the stringency of primer annealing. At lower temperatures, PCR primers can anneal to each other via regions of complementarity, and the DNA polymerase can extend the annealed primers to produce primer dimer, which often **appears** as a diffuse band of approximately 50–100bp on an ethidium bromide-stained gel. The **formation** of nonspecific products and primer-dimer can **compete** for reagent availability with amplification of the desired product. Thus, hot-start PCR can improve the yield of specific PCR products. To perform manual hot-start PCR, reactions are assembled on ice or at room temperature, but one **critical** component is **omitted until** the reaction is heated to 60–65°C, at which point the **missing** reagent is added. This omission **prevents** the polymerase from extending primers until the critical component is added at the **higher** temperature where primer annealing is more stringent. However, this method is **tedious** and **increases the risk of contamination.** A **second**, less labor-intensive approach involves the **reversible** inactivation or physical separation of one or more critical components in the reaction. For example, the **magnesium** or DNA polymerase can be sequestered in a **wax** bead, which melts as the reaction is heated to 94°C during the

denaturation step, releasing the component **only** at higher temperatures (Carothers et al. 1989; Krishnan et al. 1991; Clark, 1988). The DNA polymerase also can be kept in an **inactive** state by binding to an oligonucleotide, also known as an aptamer (Lin and Jayasena, 1997; Dang and Jayasena, 1996) or an antibody (Scalice et al. 1994; Sharkey et al. 1994). This bond is **disrupted** at the higher temperatures, releasing the functional DNA polymerase. Finally, the DNA polymerase can be **maintained** in an **inactive** state through **chemical** modification (Moretti, T. et al 1998).

Hot-Start PCR Products and Resources GoTaq® G2 Hot Start Taq is available as a **standalone enzyme or master mix,** In this formulation, the Taq polymerase is **bound** to a proprietary **antibody** that **blocks** activity. Activity is **restored** during initial denaturation, allowing hot-start PCR.

Hot Start Polymerase View Master Mixes Long-Range PCR Amplification of long DNA fragments is desirable for numerous applications such as physical mapping applications (Rose, 1991) and direct cloning from genomes. While **basic** PCR works well when **smaller** fragments are amplified, amplification efficiency (and therefore the yield of amplified fragments) **decreases** significantly as the amplicon size **increases** over 5kb. This decrease in yield can be attributed to the accumulation of truncated products, which are **not** suitable substrates for additional cycles of amplification.

These products appear as **smeared**, as opposed to discrete, bands on a gel. In 1994, Wayne Barnes (Barnes, 1994) and other researchers (Cheng et al. 1994) examined factors affecting polymerization across larger regions of DNA by thermostable DNA polymerases and identified key variables affecting the yield of **longer** PCR fragments. They devised an approach using a mixture of two thermostable polymerases to synthesize longer PCR products. The first polymerase lacks a 3′→5′ exonuclease (proofreading) activity; the second enzyme, present at a reduced concentration, contains a potent proof reading activity. Presumably, when the nonproofreading DNA polymerase (e.g., Taq DNA polymerase) **mis**incorporates a dNTP, **subsequent** extension of the newly synthesized DNA either proceeds **very slowly or stops completely.** The proofreading polymerase (e.g., Pfu DNA polymerase or Tli DNA polymerase) serves to **remove** the misincorporated nucleotide, allowing the DNA polymerases to **continue** extension of the new strand. Although the use of two thermostable DNA polymerases can significantly **increase** yield, **other conditions can have a significant impact on the yield of longer** PCR products (Cheng et al. 1995). Logically, longer extension times can increase the yield of longer PCR products because fewer partial products are synthesized. Extension times **depend** on the length of the target; times of 10–20 minutes are common. In addition, template **quality** is crucial. Depurination of the template, which is promoted

at **elevated** temperatures and **lower** pH, will result in more partial products and **decreased overall yield.** In **long** PCR, **denaturation** time is **reduced** to 2–10 seconds to **decrease** depurination of the template. **Additives**, such as **glycerol** and **dimethyl sulfoxide** (DMSO), **also help lower the strand-separation and primer-annealing temperatures, alleviating some of** the depurination effects of **high** temperatures. Cheng et al. also found that reducing **potassium** concentrations by 10–40% **increased** the amplification **efficiency** of longer products (Cheng et al. 1995). Long PCR Master Mix GoTaq® Long PCR Master MIx contains hot start Taq in a **specially formulated** mixture with a proprietary thermal stable proofreading polymerase. This **optimized** enzyme mixture allows **efficient** amplification of up to 40kb from lambda DNA **or** 30kb from human genomic DNA. View Long PCR Master Mix qPCR and RT-qPCR Quantitative Endpoint PCR PCR and RT-PCR are **generally** used in a qualitative format to **evaluate** biological samples. However, **a wide variety** of applications, such as determining **viral load**, measuring responses to **therapeutic** agents and characterizing **gene** expression, would be **improved** by quan**ti**tative determination of target abundance. **Theoretically**, this should be easy to achieve, given the **exponential** nature of PCR, **because** a linear relationship exists between the number of amplification cycles and the logarithm of the number of molecules. In **practice**, however, amplification

efficiency is **decreased** because of **contaminants** (inhibitors), competitive reactions, substrate exhaustion, polymerase inactivation and target reannealing. As the number of cycles **increases**, the amplification efficiency **decreases**, eventually **resulting** in a plateau effect. Normally, quantitative PCR requires that , measurements be taken **before** the plateau phase**??** , so that the relationship between the number of cycles and molecules is relatively linear. This point must be determined **empirically** for different reactions because of the **numerous** factors that can affect amplification efficiency. Because the measurement is taken **prior** to the reaction plateau , quantitative PCR uses **fewer** amplification cycles than **basic** PCR. This can **cause** problems in detecting the **final** product because there is **less** product to detect. To **monitor** amplification efficiency, many applications are **designed** to include an internal standard in the PCR. One **such** approach includes a second primer pair that is **specific** for a "housekeeping" gene (i.e., a gene that has constant expression levels among the samples compared) in the reaction (Gaudette and Crain, 1991; Murphy et al. 1990). Amplification of housekeeping **genes** verifies that the target nucleic acid and reaction components were of **acceptable??** quality but does **not** account for differences in amplification efficiencies due to differences in product size or primer annealing efficiency **between** the internal standard and target being quantified. The **concept** of competitive PCR

—a **variation** of quantitative PCR—is a response to this limitation. In competitive PCR, a **known** amount of a control template is added to the reaction. This template is **amplified** using the **same** primer pair as the experimental target molecule **but** yields a distinguishable product (e.g., different size, restriction digest pattern, etc.). The amounts of control and test product are compared **after** amplification. While these approaches control for the quality of the target nucleic acid, buffer components and primer annealing efficiencies, they have their own **limitations** (Siebert and Larrick, 1993; McCulloch et al. 1995), **including** the fact that **many depend on final analysis by** electrophoresis. Numerous fluorescent and solid-phase **assays** exist to measure the amount of amplification product generated in each reaction, **but they often fail to discriminate** amplified DNA of interest **from** nonspecific amplification products. Some of these analyses **rely** on **blotting** techniques, which **introduce** another variable due to nucleic acid transfer efficiencies, while **other** assays were developed to **eliminate** the need for gel electrophoresis **yet** provide the requisite **specificity??**.

Real-time PCR, which provides the ability to view the results of **each** amplification cycle, is a popular way of overcoming the need for analysis by electrophoresis.

Quantitative Real-Time PCR

The use of fluorescently labeled oligonucleotide probes or primers or fluorescent DNA-**binding dyes to detect and** quantitate a PCR product allows quantitative PCR to be performed in **real** time. **Specially designed** instruments perform both **thermal** cycling **to amplify** the target and **fluorescence** detection to monitor PCR product **accumulation**. DNA-**binding dyes** are easy to use but do **not** differentiate between specific **and** nonspecific PCR products and are **not** conducive to multiplex reactions. Fluorescently labeled nucleic acid probes have the advantage that they react with **only** specific PCR products, but they can be expensive and **difficult to design.** Some qPCR technologies employ fluorescently labeled PCR primers **instead** of probes. The use of fluorescent DNA-binding **dyes** is one of the **easiest** qPCR approaches. The **dye** is simply added to the reaction, and fluorescence is measured at **each** PCR cycle. Because fluorescence of these dyes **increases** dramatically in the presence of double-stranded? DNA, DNA synthesis can be monitored **as an increase** in fluorescent signal. **However**, preliminary work often **must** be done to ensure that the PCR conditions yield **only specific** product. In **subsequent** reactions, **specific** amplification can **verified by a melt curve** analysis. Thermal melt curves are generated **by** allowing **all** product **to form??** double-stranded DNA at a **lower** temperature (approximately 60°C) and **slowly** ramping the temperature to **de**naturing levels (approximately 95°C). The product length and

sequence **affect** melting temperature (Tm), so the melt curve is **used** to characterize amplicon homogeneity. Nonspecific amplification can be **identified** by broad peaks in the melt curve or peaks with unexpected Tm values. By distinguishing specific **and** nonspecific amplification products, the melt curve adds a quality control aspect during routine use. The generation of melt curves is **not** possible with assays that rely on the 5′→3′ exonuclease activity of Taq DNA polymerase, such as the probe-based TaqMan® technology. Some qPCR strategies employ complementary nucleic acid probes to **quantify?** the DNA target. These probes also can be used to detect single nucleotide polymorphisms (Lee et al. 1993; Bernard et al. 1998). There are **several** general categories of real-time PCR probes, including hydrolysis, hairpin and simple hybridization probes. These probes contain a **complementary** sequence that allows the probe to **anneal** to the accumulating PCR product, but probes can **differ** in the number and location of the fluorescent reporters. Hydrolysis probes are labeled with a fluor at the 5′-**end??** and a quencher at the 3′-**end??**, and because the two reporters are in close proximity, the fluorescent signal is quenched. During the **annealing** step, the probe hybridizes to the PCR product generated in previous amplification cycles. The **resulting** probe:target hybrid is a substrate for the 5′→3′ exonuclease activity of the DNA polymerase, which degrades the annealed probe and liberates the fluor (Holland et al. 1991). The fluor is freed

from the effects of the energy-absorbing quencher, and the progress of the reaction and accumulation of PCR product is monitored by the **resulting** increase in fluorescence. With this approach, preliminary experiments must be performed **prior** to the quantitation experiments to show that the signal generated is proportional to the amount of the desired PCR product and that **non**specific amplification does **not** occur. **Hairpin** probes, **also** known as **molecular** beacons, contain **inverted repeats** separated by a sequence complementary to the target DNA. The repeats anneal to **form** a hairpin structure, where the fluor at the 5′-end and a quencher at the 3′-end are in close proximity, resulting in **little** fluorescent signal. The hairpin probe is designed so that the probe binds **preferentially?** to the target DNA **rather** than **retains** the hairpin structure. As the reaction progresses, increasing amounts of the probe , anneal to the accumulating PCR product, and as a result, the fluor and quencher become physically **separated??**. The fluor is no longer quenched, and the **level** of fluorescence increases. One **advantage** of this technique is that hairpin probes are **less** likely to **mismatch** **than** hydrolysis probes (Tyagi et al. 1998). However, preliminary experiments must be performed to show that the signal is **specific?** for the desired PCR product and that **non**specific amplification does **not** occur. The use of simple hybridization probes involves **two** labeled probes or, **alternatively**, one labeled probe **and** a labeled PCR

primer. In the **first** approach, the energy emitted by the fluor on one probe is **absorbed** by a fluor on the second probe, which hybridizes nearby. In the second approach, the emitted energy is **absorbed** by a second fluor that is incorporated into the PCR product as part of the primer. Both of these approaches **result** in increased fluorescence of the energy acceptor **and** decreased fluorescence of the energy donor. The use of hybridization probes can be **simplified** even further so that **only** one labeled probe is required. In this approach, quenching of the fluor by deoxy**guanosine** is used to bring about a change in fluorescence (Crockett and Wittwer, 2001; Kurata et al. 2001). The labeled probe anneals so that the fluor is in close proximity to G residues within the target sequence, and as probe annealing increases, fluorescence decreases due to deoxyguanosine quenching. With this approach, the location of probe is **limited** , because the probe must hybridize so that the fluorescent **dye** is very near a G residue. The advantage of simple hybridization probes is their ability to be multiplexed **more easily** than hydrolysis and hairpin probes through the use of differently colored fluors and probes with different melting temperatures (reviewed in Wittwer et al. 2001). Some qPCR strategies employ complementary nucleic acid probes to quantify the DNA target. These probes **also** can be used to detect single nucleotide polymorphisms (Lee et al. 1993; Bernard et al. 1998). There are **several** general categories of real-time PCR probes, including hydrolysis, hairpin

and simple hybridization probes. These probes contain a complementary sequence that allows the probe to anneal to the accumulating PCR product, but probes can differ in the number and location of the fluorescent reporters. // qPCR Products and Resources GoTaq® qPCR and RT-qPCR kits are available for dye-based or probe-based real-time PCR approaches. GoTaq qPCR Systems contain BRYT **Green** Dye, which provides maximum amplification efficiency and greater fluorescence than SYBR Green. GoTaq® Probe Systems are **ready-to-use master mixes** that simplify reaction assembly for hydrolysis probe-based detection.

General Considerations for PCR Optimization
This discussion focuses on the use of Taq DNA polymerase in PCR, since **this is the enzyme most commonly used** in PCR. Many of these suggestions also apply when using **other** DNA polymerases.
Magnesium Concentration… Magnesium is a required **cofactor** for thermostable DNA polymerases, and magnesium concentration is a **crucial** factor that can **affect** amplification success. Template DNA concentration, chelating **agents** present in the sample (e.g., EDTA or citrate), dNTP concentration and the presence of proteins **all** can affect the amount of **free** magnesium in the reaction. In the **absence** of adequate **free** magnesium, Taq DNA polymerase is **inactive**. **Excess** free magnesium **reduces** enzyme fidelity (Eckert

and Kunkel, 1990) and may **increase** the level of **non**specific amplification (Williams, 1989; Ellsworth et al. 1993). For these reasons, researchers should empirically determine the **optimal** magnesium concentration for each target. To do so, set up a **series** of reactions containing 1.0–4.0mM Mg2+ in 0.5–1mM increments **and** visualize**??** the results to determine which magnesium concentration produced the highest**??** yield of product **and** the **minimal** amount of **non**specific product. The effect of magnesium concentration **and** the optimal concentration **range** can **vary** with the particular DNA polymerase. For example, the performance of Pfu DNA polymerase seems depend **less** on magnesium concentration, but when optimization is required, the optimal concentration is usually in the range of 2–6mM. Many DNA polymerases are supplied with a magnesium-free reaction buffer and a tube of 25mM MgCl2 so that you can **adjust** the Mg2+ concentration to the optimal level for **each** reaction. Before assembling the reactions, be sure to **thaw** the **magnesium** solution completely prior to use and **vortex** the magnesium solution for several seconds before pipetting. Magnesium chloride solutions can **form** concentration gradients as a result of multiple freeze-thaw cycles, **and** vortex mixing is required to obtain a uniform solution. These **two** steps, though seemingly simple, **eliminate??** the cause of many failed experiments. Some scientists prefer to use reaction buffers that **already** contain MgCl2 at a final concentration of

1.5mM. It should be noted, however, that Hu et al. (1992) reported performance **variability** of reaction buffer solutions containing magnesium. The **free** magnesium changes of 0.6mM observed in their experiments **dramatically** affected amplification yields in an allele-specific manner. The authors found that **heating** the buffer at 90°C for 10 minutes **restored** the homogeneity of the solution. They **postulated!!!!** that magnesium chloride precipitates as a result of multiple freeze-thaw cycles.

Effect of magnesium concentration on PCR
Effects of magnesium concentration on amplification. Amplifications were performed using **various** Mg concentrations to demonstrate the effect on the amplification of a 1.8kb target luciferase gene. The reaction products were **analyzed??** by agarose gel electrophoresis **followed by ethidium bromide staining.** Lane M, Promega pGEM® DNA Markers (Cat.# G1741); lane 1, 0mM Mg^{2+}; lane 2, 0.5mM Mg^{2+}; lane 3, 1mM Mg^{2+}; lane 4, 1.5mM Mg^{2+}; lane 5, 2mM Mg^{2+}; lane 6, 2.5mM Mg^{2+}; lane 7, 3mM Mg^{2+} and lane 8, 3.5mM Mg^{2+}.

Buffer Considerations
Most reaction buffers consist of a buffering **agent**, most often a Tris-based buffer, and **salt**, commonly KCl. The buffer regulates the pH of the reaction, which affects

DNA polymerase activity and fidelity. Modest concentrations of KCl will **increase** DNA polymerase activity by 50–60% over activities in the absence of KCl; 50mM KCl is considered optimal (Gelfand, 1989). GoTaq® DNA Polymerase contains native Taq DNA polymerase in a proprietary formulation. It is supplied with 5X Green GoTaq® Reaction Buffer and 5X Colorless GoTaq® Reaction Buffer. The 5X Green GoTaq® Reaction Buffer contains blue **and** yellow dyes that separate during electrophoresis to monitor migration progress. The buffer **also** contains a compound that increases the **density** of the sample so that it will sink into the well of the agarose gel, allowing reactions to be directly loaded onto an agarose gel without the need for loading dye. The blue dye co-migrates at the same rate as a 3–5kb DNA fragment in a 1% agarose gel. The yellow dye migrates at a rate faster than primers (<50bp) in a 1% agarose gel. The 5X Colorless GoTaq® Reaction Buffer and 5X Green GoTaq® Reaction Buffer have the same formulation, except for the dyes. The 5X Colorless GoTaq® Reaction Buffer is **recommended** for any applications where absorbance **or** fluorescence measurements of the PCR amplimer? will be taken **without** prior cleanup. Both buffers are supplied at pH 8.5 and contain MgCl2 at a concentration of 7.5mM for a final concentration of 1.5mM. GoTaq® Flexi DNA Polymerase is supplied with 5X Green GoTaq® Flexi Reaction Buffer and 5X Colorless GoTaq® Flexi

Reaction Buffer. The compositions are **identical** to the 5X Green GoTaq® Reaction Buffer and 5X Colorless GoTaq® Reaction Buffer, except that the GoTaq® Flexi reaction buffers do not contain MgCl2. Instead, the GoTaq® Flexi DNA Polymerase is supplied with a tube of 25mM MgCl2 so that reactions can be supplemented with varying concentrations of **magnesium**.

Enzyme Concentration

We recommend using 1–1.25 units of Taq DNA polymerase in a 50µl amplification reaction. In most cases, this is an **excess** of enzyme, and adding more enzyme will **not** significantly increase product yield. In fact, increased amounts of enzyme increase the likelihood of generating **artifacts** associated with the intrinsic 5′→3′ exonuclease activity of Taq DNA polymerase, resulting in smeared bands in an agarose gel (Longley et al. 1990; Bell and DeMarini, 1991). Pipetting errors are a frequent **cause** of **excessive** enzyme levels. Accurate dispensing of small volumes of enzyme solutions in 50% glycerol is **difficult**, so we strongly recommend preparing a reaction **master** mix, which requires a **larger** volume of each **reagent**, **to reduce** pipetting errors.

PCR Primer Design

PCR primers **define** the target region to be amplified and generally range in length from 15–30 bases. Ideally primers will have a GC-content of 40–60%. Avoid three

G or C residues in a row near the 3′-end of the primer to **minimize** **non**specific primer annealing. Also, **avoid** primers with intra- or intermolecular complementary sequences to **minimize** the production of primer-dimer. **Intra**molecular regions of secondary structure can **interfere** with primer annealing to the template and should be avoided. Ideally, the melting temperature (Tm), the temperature at which 50% of the primer molecules are annealed to the complementary sequence, of the two primers will be within 5°C so that the primers anneal efficiently at the same temperature. Primers can be **designed** to include sequences that are useful for downstream applications. For example, **restriction enzyme sites** can be placed at the 5′-ends of PCR primers to facilitate subsequent cloning of the PCR product, or a T7 RNA polymerase promoter can be added to allow in vitro transcription without the need to subclone the PCR product into a vector.

Template Quality

Successful amplification depends on DNA template quantity **and** quality. **Reagents** commonly used to **purify** nucleic acids (salts, guanidine, proteases, organic solvents and SDS) are **potent inactivators** of DNA polymerases. For example, 0.01% SDS will **inhibit** Taq DNA polymerase by **90%**, while 0.1% SDS will **inhibit** Taq DNA polymerase by **99.9%** (Konat et al. 1994). A few **other** examples of PCR **inhibitors** are **phenol** (Katcher

and Schwartz, 1994), **heparin** (Beutler et al. 1990; Holodniy et al. 1991), **xylene cyanol, bromophenol blue** (Hoppe et al. 1992), **plant polysaccharides** (Demeke and Adams, 1992), and the **polyamines spermine and spermidine** (Ahokas and Erkkila, 1993). In **some** cases, the **inhibitor** is **not** introduced into the reaction with the nucleic acid template. A **good** example of this is , an inhibitory substance that can be released from polystyrene or polypropylene upon exposure to **ultraviolet** light (Pao et al. 1993; Linquist et al. 1998). If an amplification reaction **fails** and you suspect the DNA template is **contaminated** with an inhibitor, **add** the suspect DNA preparation to a control reaction with a DNA template and primer pair that has amplified well in the past . Failure to amplify the control DNA **usually** indicates the presence of an **inhibitor**. Additional steps to **clean up** the DNA preparation, such as **phenol:chloroform** extraction or **ethanol** precipitation, may be necessary.

Template Quantity

The amount of template required for successful amplification **depends** upon the **complexity** of the DNA sample. For example, of a 4kb plasmid containing a 1kb target sequence, 25% of the input DNA is the target of interest. Conversely, a 1kb target sequence in the human genome (3.3 × 109bp) **represents** approximately 0.00003% of the input DNA. Thus, approximately

1,000,000-**fold** more human genomic DNA is required to maintain the same **number** of target copies per reaction. **Common mistakes include** using too much plasmid DNA, too much PCR product or too little genomic DNA as the template. Reactions with too little DNA template will have low yields, while reactions with too much DNA template can be plagued by nonspecific amplification. If possible, **start** with >104 copies of the target sequence to obtain a signal in 25–30 **cycles**, but try to keep the **final** DNA concentration of the reaction ≤10ng/µl.
When **reamplifying** a PCR product, the concentration of the specific PCR product is often **not** known. We recommend **diluting** the previous amplification reaction 1:10 to 1:10,000 **before** reamplification. 1µg of 1kb RNA = 1.77 × 1012 molecules 1µg of 1kb dsDNA = 9.12 × 1011 molecules 1µg of pGEM® Vector DNA = 2.85 × 1011 molecules 1µg of lambda DNA = 1.9 × 1010 molecules 1µg of E. coli genomic DNA = 2 × 108 molecules 1µg of human genomic DNA = 3.04 × 105 molecules .

Cycling Parameters
The **two most** commonly **altered** cycling parameters are annealing temperature **and** extension time. The lengths and temperatures for the **other** steps of a PCR cycle do **not** usually vary significantly. However in some cases, the **de**naturation cycle can be shortened **or** a lower denaturation temperature used to reduce the number of

depurination events, which can lead to **mutations** in the PCR products. Primer sequence is a **major** factor that determines the optimal annealing temperature, which is often within 5°C of the melting temperature of the primers. Using an annealing temperature slightly higher than the primer Tm will increase annealing stringency and can minimize nonspecific primer annealing and decrease the amount of **undesired?** products synthesized. Using an annealing temperature **lower** than the primer Tm can result in **higher** yields, as the primers anneal more efficiently at the lower temperature. We recommend testing **several** annealing temperatures, starting approximately 5°C below the Tm, to determine the best annealing conditions. In **many** cases, **non**specific amplification and primer-dimer formation can be reduced through optimization of annealing temperature, **but if** undesirable PCR products remain a problem, consider incorporating one of the many hot-start PCR methods. Oligonucleotide synthesis facilities will often provide an estimate of a primer's Tm.

The Tm also can be calculated using the **Biomath** Calculators.
Numerous formulas exist to determine the **theoretical** Tm of nucleic acids (Baldino, Jr. et al. 1989; Rychlik et al. 1990).

The **formula** below can be used to **estimate** the melting temperature for oligonucleotides: Tm = 81.5 + 16.6 × (log10[Na+]) + 0.41 × (%G+C) – 675/n where [Na+] is the molar salt concentration and n = number of bases in the oligonucleotide Example. To **calculate** the melting temperature of a 22mer oligonucleotide with 60% G+C in 50mM KCl: Tm = 81.5 + 16.6 × (log10[0.05]) + 0.41 × (60) – 675/22 = 81.5 + 16.6 × (–1.30) + 24.60 – 30.68 = 54°C

The length of the extension cycle, which may need to be optimized, **depends** on PCR product size and the DNA polymerase being used. In general, allow approximately 1 minute?? for **every** 1kb of amplicon (minimum extension time = 1 minute) for nonproofreading DNA polymerases and 2 minutes for every 1kb of amplicon for proofreading DNA polymerases. **Avoid excessively** long extension times, as they can **increase** the likelihood of generating artifacts associated with the intrinsic 5′→3′ exonuclease activity of Taq DNA polymerase (Longley et al. 1990; Bell and DeMarini, 1991). PCR typically involves 25–35 **cycles** of amplification. The **risk** of **un**desirable PCR products appearing in the reaction increases **as** the cycle number increases, so we recommend performing only enough**???** cycles to synthesize the desired amount of product. **If non**specific amplification products accumulate **before** sufficient amounts of PCR product can be synthesized, consider **diluting** the products of the **first** reaction and performing a second amplification with the

same primers or primers that anneal to sequences within the **desired** PCR product (nested primers).

PCR **Enhancers** and **Additives**

Addition of PCR-enhancing agents can increase yield of the desired PCR product or **decrease** production of undesired products. There are **many** PCR enhancers, which can act through a **number** of **different** mechanisms. These reagents will **not** enhance **all** PCRs; the beneficial effects are **often** template- and primer-specific **and** will need to be determined **empirically**. Some of the more common **enhancing** agents are discussed below. Addition of betaine, DMSO and formamide can be helpful when amplifying GC-rich templates and templates that form strong **secondary** structures, which can cause DNA polymerases to stall. GC-rich templates can be **problematic** due to **inefficient** separation of the two DNA strands**???** or the tendency for the complementary, GC-rich primers to form **inter**molecular secondary structures, which will compete with primer annealing to the template. **Betaine** reduces the amount of energy required to separate**??** DNA strands (Rees et al. 1993). DMSO and formamide are **thought to** aid amplification in a similar manner by interfering with **hydrogen** bond formation between two DNA strands**??** (Geiduschek and Herskovits, 1961). Some reactions that amplify poorly in the **absence** of enhancers will give a **higher** yield of PCR product when betaine (1M), DMSO

(1–10%) or formamide (1–10%) are added. Concentrations of DMSO greater than 10% and formamide greater than 5% can **inhibit** Taq DNA polymerase **and** presumably other DNA polymerases as well (Varadaraj and Skinner, 1994). In some cases, general stabilizing agents such as BSA (0.1mg/ml), **gelatin** (0.1–1.0%) and non-ionic **detergents** (0–0.5%) can **overcome** amplification failure. These additives can increase DNA polymerase stability and reduce the loss of reagents through adsorption to tube walls. BSA also has been shown to **overcome** the **inhibitory** effects of **melanin** on RT-PCR (Giambernardi et al. 1998). Non-ionic detergents, such as Tween®-20, NP-40 and Triton® X-100, have the **added** benefit of overcoming inhibitory effects of trace amounts of strong ionic detergents, such as 0.01% SDS (Gelfand and White, 1990). **Ammonium** ions can make an amplification reaction more tolerant of **non**optimal conditions. For this reason, some PCR **reagents** include 10–20mM $(NH_4)_2SO_4$. **Other** PCR enhancers include **glycerol** (5–20%), **polyethylene glycol** (5–15%) and **tetramethyl ammonium chloride** (60mM).

Nucleic Acid Cross-Contamination

It is important to minimize cross-contamination between samples and prevent carryover of RNA and DNA from one experiment to the next. Use separate work areas and pipettors for pre- and post-amplification steps. Use

positive displacement pipettes or aerosol-resistant tips to reduce cross-contamination during pipetting. Wear gloves, and change them often. There are a number of techniques that can be **used to prevent** amplification of contaminating DNA. PCR reagents can be treated with **isopsoralen**, a photo-activated, cross-linking reagent that intercalates into double-stranded DNA molecules and forms covalent, interstrand crosslinks, to **prevent** DNA denaturation and **replication**. These inter-strand crosslinks effectively render contaminating DNA **un**amplifiable. Treatment of PCR reagents with **uracil**-N-glycosylase (UNG), a DNA **repair enzyme** that **hydrolyzes** the base-ribose bond at uracil residues, **eliminates** one of the most common sources of DNA contamination: **previously** amplified PCR products. UNG treatment **prevents** replication of uracil-containing DNA , by **causing** the DNA polymerase to stall at the resulting **abasic** sites. For UNG to be an effective safeguard **against** contamination, the products of **previous** amplifications must be synthesized in the presence of dUTP. This is easily accomplished by substituting dUTP for some or all of the dTTP in the reaction. **Non**proofreading polymerases will readily incorporate dUTP into a PCR product, **but** proofreading polymerases incorporate dUTP much **less** efficiently (Slupphaug et al. 1993; Greagg et al. 1999; Lasken et al. 1996).

Since dUTP incorporation has **no** noticeable effect on the intensity of ethidium bromide staining or electrophoretic mobility of the PCR product, reactions can be **analyzed** by standard agarose gel electrophoresis. While both methods are effective**??** (Rys and Persing, 1993), UNG treatment has the advantage that **both** single-stranded and double-stranded DNA templates will be rendered **un**amplifiable (Longo et al. 1990).

General Considerations for RT-PCR
Please also read General Considerations for PCR Optimization (above) . Many of the important parameters discussed there **also** apply to RT-PCR. For a discussion of **reverse** transcriptases commonly used for RT-PCR, see the Thermostable Polymerases and Reverse Transcriptases section (below).

Template Considerations
For RT-PCR, successful reverse transcription **depends** on RNA integrity and purity. Procedures for creating and maintaining a ribonuclease-free (RNase-free) **environment** to minimize RNA degradation are described in Blumberg, 1987. The use of an RNase **inhibitor** (e.g., Recombinant RNasin® Ribonuclease Inhibitor) is **strongly** recommended. For **optimal** results, the RNA template, whether a total RNA preparation, an mRNA population or a synthesized RNA transcript, should be DNA-**free** to avoid amplification of contaminating DNA.

The **most** commonly used DNA polymerases for PCR have **no** reverse transcriptase activity under **standard** reaction conditions, and thus, amplification products will be generated **only if** the template contains **trace** amounts of DNA with similar sequences. Successful RT-PCR **also depends** on RNA **quantity**, which may need to be varied to determine the optimal amount. Excellent amplification results can be obtained with the Access and AccessQuick™ RT-PCR Systems using total RNA template levels in the range of 1pg–1µg per reaction (Figure 3) or poly(A)+ RNA template levels in the range of 1pg–100ng.

Amplification of a specific message in total RNA.
RT-PCR amplifications containing the **indicated** amounts of mouse liver total RNA were performed using the Access RT-PCR System as described in the using oligonucleotide primers specific to the mouse β-actin transcript. The specific 540bp amplicon is indicated. Equivalent aliquots of each amplification reaction were separated on a 3% NuSieve®/ 1% agarose gel in 1X TAE buffer containing 0.5µg/ml **ethidium** bromide. Lanes M, 100bp DNA Ladder (Cat.# G2101).

Reverse Transcription Primer Design
Selection of an appropriate primer for reverse transcription **depends** on target mRNA size and the presence of **secondary** structure. For example, a primer

that anneals specifically to the 3′-end of the transcript (a sequence-specific primer or oligo(dT) primer) may be **problematic** when reverse transcribing the 5′-ends of long mRNAs or molecules that have significant secondary structure, which can **cause** the reverse transcriptase to stall during cDNA synthesis. Random hexamers prime reverse transcription at multiple points along the transcript. For this reason, they are useful for either long mRNAs or transcripts with significant secondary structure. Whenever possible, we recommend using a primer that anneals **only** to , **defined** sequences in particular RNAs (sequence-specific primers) **rather** than to entire RNA populations in the sample (e.g., random hexamers or oligo(dT) primer). To **differentiate** between amplification of cDNA and amplification of contaminating genomic DNA, **design** primers to anneal to sequences in exons on opposite sides of an intron , so that any amplification product **derived from** genomic DNA will be **much** larger than the product amplified **from** the target cDNA. This **size difference** not only makes it possible to differentiate the two products by gel electrophoresis **but** also favors the synthesis of the smaller cDNA-derived product (amplification of smaller fragments is often **more** efficient**?** than that of long fragments). Regardless of primer choice, the **final** primer concentration in the reaction is usually **within** the range of 0.1–1.0μM, but this **may** need to be optimized. We recommend using a **final** concentration of 1μM primer

(50pmol in a 50µl reaction) as a **starting** point for optimization. More information on PCR primer design is provided in the PCR Primer Design section.

Cycle Parameters

Efficient first-strand cDNA synthesis can be accomplished in a 20- to 60-minute **incubation** at 37–45°C **using** AMV reverse transcriptase or at 37–42° for M-MLV reverse transcriptase. When using AMV RT we recommend using a sequence-specific primer and performing reverse transcription at 45°C for 45 minutes as a starting point. The **higher** reaction temperature will minimize the effects of RNA secondary structure and encourage full-length cDNA synthesis. First-strand cDNA synthesis with random hexamers and oligo(dT) primer should be conducted at **room** temperature (20–25°C) **and** 37°C, respectively. The Access and AccessQuick™ RT-PCR Systems do **not** require RNA denaturation prior to initiation of the reverse transcription reaction. **If desired,** however, a denaturation step may be **incorporated** by incubating a **separate** tube containing the primers and RNA template at 94°C for 2 minutes. Do **not** incubate AMV reverse transcriptase at 94°C; it will be **inactivated**. The template/ primer mixture then can be **cooled** to 45°C and added to the RT-PCR **mix** for the standard reverse transcription incubation at 45°C. Following the reverse transcription, we **recommend** a 2-minute incubation at

94°C to denature the RNA/cDNA hybrid, **inactivate** AMV reverse transcriptase and **dissociate** AMV RT from the cDNA. It has been **reported** that AMV reverse transcriptase must be **inactivated** to obtain **high** yields of amplification product (Sellner et al. 1992; Chumakov, 1994). **Most** RNA samples can be detected using 30–40 cycles of amplification. If the target RNA is **rare or if only a small** amount of starting material is available, it may be necessary to increase the number of cycles to 45 or 50 **or dilute** the products of the first reaction and **re**amplify.

Thermostable Polymerases and Reverse Transcriptases
Thermostable DNA Polymerases
Prior to the use of thermostable DNA polymerases in PCR, researchers had to **laboriously** replenish the reaction with **fresh** enzyme (such as Klenow or T4 DNA polymerase) after **each** denaturation cycle. Thermostable DNA polymerases **revolutionized** and **popularized** PCR because of their ability to **withstand** the high denaturation temperatures. The use of thermostable DNA polymerases also **allowed** higher annealing temperatures, which improved the stringency of primer annealing.
Thermostable DNA polymerases can be used for either one-enzyme **or** two-enzyme RT-PCR (Myers and Gelfand, 1991; Chiocchia and Smith, 1997). For example, Tth DNA polymerase can **act as a reverse** transcriptase in the presence of Mn2+ for **one**-enzyme RT-PCR (Myers

and Gelfand, 1991). All of the DNA polymerases mentioned below can be used to amplify first-strand cDNA produced by a reverse transcriptase, such as AMV RT, in two-enzyme RT-PCR. Thermostable DNA polymerases can be **divided into two groups**: those with a 3′→5′ exonuclease (proofreading) activity, such as Pfu DNA polymerase, **and** those without the proofreading function, such as Taq DNA polymerase. These two groups have some important **differences**. Proofreading DNA polymerases are **more accurate** than nonproofreading polymerases due to the 3′→5′ exonuclease activity, which can **remove** a misincorporated nucleotide from a growing DNA chain. When the amplified product is to be **cloned**, expressed or used in mutation analysis, Pfu DNA polymerase is a **better** choice due to its **high** fidelity. However, for **routine** PCR, where simple detection of an amplification product is the goal, Taq DNA polymerase is the most commonly used enzyme because yields tend to be higher with a nonproofreading DNA polymerase. Amplification with nonproofreading DNA polymerases **results** in the template-independent addition of a single nucleotide to the 3′-end of the PCR product, whereas the use of proofreading DNA polymerases **results** in blunt-ended PCR products (Clark, 1988; Hu, 1993). The single-nucleotide overhang can simplify the cloning of PCR products. Proofreading DNA polymerases **also** are used in blends with **non**proofreading DNA polymerases, or

amino-terminally truncated **versions** of Taq DNA polymerase, to amplify longer stretches of DNA with greater accuracy than the nonproofreading DNA polymerase alone (Barnes, 1994; Cline et al. 1996).

Taq DNA Polymerase

Taq DNA polymerase is isolated **from** Thermus aquaticus and catalyzes the primer-dependent incorporation of nucleotides into duplex DNA in the 5′→3′ direction in the presence of Mg2+. The enzyme does **not** possess 3′→5′ exonuclease activity but **has** 5′→3′ exonuclease activity. Taq DNA polymerase is suitable for most PCR applications that do **not** require a high-fidelity enzyme, such as detecting specific DNA or RNA sequences. The **error rate** of Taq DNA polymerase is approximately 1 × 10–5 errors/base, although the **fidelity does depend somewhat on the reaction conditions.** The fidelity is **slightly** higher at **lower** pH, **lower** magnesium concentration and relatively **low** dNTP concentration (Eckert and Kunkel, 1990; Eckert and Kunkel, 1991). Taq DNA polymerase is commonly used to amplify PCR products of 5kb or less. PCR products in the range of 5–10kb can be amplified with Taq DNA polymerase but often **require more** optimization than smaller PCR products. For products larger than approximately 10kb, we recommend an enzyme **or** enzyme **mix** and reaction **conditions** that are **designed** for long PCR. Taq DNA polymerase is a processive **enzyme** with an extension rate

of >60 nucleotides/second at 70°C (Innis et al. 1988), so an extension step of 1 minute per 1kb to be amplified should be **sufficient** to generate full-length PCR products. The enzyme has a **half**-life of 40 minutes at 95°C (Lawyer et al. 1993). Because Taq DNA polymerase is a nonproofreading polymerase, PCR products generated with Taq DNA polymerase **will** contain a single-nucleotide 3′ overhang, usually a 3′ A overhang.

Tfl DNA Polymerase

Tfl DNA polymerase **catalyzes** the primer-dependent polymerization of nucleotides into duplex DNA in the presence of Mg2+. In the presence of Mn2+, Tfl DNA polymerase can use RNA as a template. Tfl DNA polymerase exhibits a 5′→3′ exonuclease activity but lacks a 3′→5′ exonuclease activity. This enzyme is commonly used in PCR (Gaensslen et al. 1992), where its activity is **similar** to that of Taq DNA polymerase. Tfl DNA polymerase is used in the Access and AccessQuick™ RT-PCR Systems.

Tth DNA Polymerase

Tth DNA polymerase catalyzes polymerization of nucleotides into duplex DNA in the 5′→3′ direction in the presence of MgCl2. The enzyme can use an RNA template in the presence of MnCl2 (Myers and Gelfand, 1991; Ruttimann et al. 1985). Tth DNA polymerase exhibits a 5′→3′ exonuclease activity but lacks detectable

3′→5′ exonuclease activity. The **error rate** of Tth DNA polymerase has been measured at 7.7 × 10–5 errors/base (Arakawa et al. 1996). Tth DNA polymerase can amplify target DNA in the presence of phenol-saturated buffer (Katcher and Schwartz, 1994) and has been reported to be **more resistant** to inhibition by blood components than other thermostable polymerases (Ehrlich et al. 1991; Bej and Mahbubani, 1992). Tth DNA polymerase is commonly used for PCR (Myers and Gelfand, 1991; Carballeira et al. 1990) **and** RT-PCR (Myers and Gelfand, 1991; Chiocchia and Smith, 1997). For primer extension, RT-PCR and cDNA synthesis using RNA templates with complex secondary structure, the **high** reaction temperature of Tth DNA polymerase **may** be an advantage **over** , more commonly used reverse transcriptases, **such as** AMV and M-MLV reverse transcriptases. **Recombinant** Tth DNA polymerase has been shown to exhibit RNase H-like activity (Auer et al. 1995).

Pfu DNA Polymerase

Pfu DNA polymerase has one of the **lowest error** rates of all known thermophilic DNA polymerases used for amplification due to the high 3′→5′ exonuclease activity (Cline et al. 1996; Andre et al. 1997). For cloning and expressing DNA after PCR, Pfu DNA polymerase is often the enzyme of **choice**. Pfu DNA polymerase can be used alone to amplify DNA fragments up to 5kb by **increasing**

the extension time to 2 minutes per kilobase. It is also used in **blends** with DNA polymerases **lacking** the proofreading function, such as Taq DNA polymerase, to achieve longer amplification products **than** with Pfu DNA polymerase alone (Barnes, 1994). However, the proofreading activity can **shorten** PCR primers, leading to decreased yield **and** increased nonspecific amplification.

Reverse Transcriptases

The discovery**???** of reverse transcriptases, **or** RNA-dependent DNA polymerases, and their role in retrovirus infection (Baltimore, 1970; Temin and Mizutani, 1970) **altered** molecular biology's central dogma of DNA→RNA→protein. Reverse transcriptases use an RNA template to synthesize DNA and **require** a primer for synthesis, like other DNA polymerases. For in **vitro** applications, the primer can be **either** oligo(dT), which hybridizes to the poly(A)+ tails of eukaryotic mRNAs, random hexamers, which prime synthesis throughout the length of the RNA template, or a sequence-specific primer, which hybridizes to a known sequence within the RNA template. Polymerization from a primer then proceeds as for DNA-dependent DNA polymerases. The commonly used reverse transcriptases, AMV reverse transcriptase, and M-MLV reverse transcriptase, 'M-MLV reverse transcriptase,RNase H minus', perform the **same** reaction but at **different** optimum temperatures

(AMV, 42°C; M-MLV, 37°C; and M-MLV RT, 'M-MLV RT, RNase' H–, 42°C). **Some** reverse transcriptases also possess intrinsic 3′- or 5′-exoribonuclease (RNase) activity, which is generally used to **degrade** the RNA template after first strand cDNA synthesis. Absence of the 5′-exoribonuclease (RNase H) activity **may** aid production of longer cDNAs (Berger et al. 1983). Some DNA-dependent DNA polymerases **also** possess a reverse transcriptase activity, which can be favored under certain conditions. For example, the thermostable, DNA-dependent Tth DNA polymerase exhibits reverse transcriptase activity when Mn2+ is substituted for Mg2+ in a reaction.

AMV Reverse Transcriptase

AMV RT catalyzes DNA polymerization using template DNA, RNA or RNA:DNA hybrids (Houts et al. 1979). AMV reverse transcriptase is the **preferred** reverse transcriptase for templates with **high** secondary structure due to its **higher** reaction temperature (up to 58°C). AMV RT is used in a **wide** variety of applications including cDNA synthesis (Houts et al. 1979; Gubler and Hoffman, 1983), RT-PCR and rapid amplification of cDNA ends (RACE; Skinner et al. 1994). Although the **high** optimal temperature (42°C) makes it the enzyme of choice for cDNA synthesis using templates with complex secondary structure, its relatively high RNase H activity **limits** its usefulness for generation of long cDNAs (>5kb). For

these templates, ‘M-MLV RT, RNase H minus’, may be a better choice.

M-MLV Reverse Transcriptase
M-MLV RT is a **single**-polypeptide, RNA-dependent DNA polymerase. The enzyme **also** has DNA-dependent DNA polymerase activity at high enzyme levels (Roth et al. 1985). M-MLV RT is used in a **variety** of applications, including cDNA synthesis, RT-PCR and RACE (Gerard, 1983). Its relatively **low** RNase H activity compared to AMV RT makes M-MLV RT the enzyme of choice for generating long cDNAs (>5kb) (Sambrook and Russell, 2001). However, for short templates with complex secondary structure, AMV RT or ‘M-MLV RT, RNase H minus’, may be a better choice due to their higher optimal temperatures. M-MLV RT is **less processive** than AMV RT, so **more** units of M-MLV RT may be **required** to generate the same amount of cDNA (Schaefer, 1995).

M-MLV Reverse Transcriptase, RNase H Minus
M-MLV reverse transcriptase, RNase H minus, is an RNA-dependent, 5′→3′ DNA polymerase that has been genetically **altered** to **remove** the associated ribonuclease H activity, which **causes** degradation of the RNA strand of an RNA:DNA hybrid (Tanese and Goff, 1988). The **absence** of RNase H activity makes M-MLV, RNase H minus, the enzyme of choice for generating long cDNAs

(>5kb). However, for shorter templates with complex secondary structure, AMV reverse transcriptase may be a better choice because it can be used at **higher** reaction temperatures. There are **two** forms of M-MLV, RNase H minus: the deletion mutant **and** the point mutant. As the names suggest, the **deletion** mutant had a specific sequence in the RNase H domain **deleted**, and the **point** mutant has a **point** mutation **introduced** in the RNase H domain. While the native M-MLV RT has a recommended reaction temperature of 37°C, the deletion and point mutants are **more** stable at higher temperatures and can be used at reaction temperatures of up to 50°C and 55°C, respectively, **depending** upon the reverse transcription **primers** used. The **point** mutant is often preferred over the deletion mutant because the point mutant has DNA polymerase activity comparable to that of the wildtype M-MLV **enzyme**, whereas the deletion mutant has a **slightly** reduced DNA polymerase activity compared to that of the wildtype enzyme.
Comparison of the mass amount of total cDNA synthesized from 2µg of a 7.5kb RNA template by increasing amounts of three Promega M-MLV reverse transcriptases. Each first-strand cDNA reaction was performed using 2µg of a 7.5kb RNA template (1µl), 0.5µg of oligo(dT)15 primer (1µl) and 14µl water. The RNA and oligo(dT)15 primer were heated at 70°C for 5 minutes and cooled on ice for 5 minutes. Five microliters of M-MLV RT 5X Buffer, 1.25µl of 10µM dNTPs, 0.5µl

of α-32P dCTP (10µCi/µl, 400Ci/mmol) and either 25, 50, 100, 150, 200 or 400 units of M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant; M-MLV Reverse Transcriptase, RNase H Minus, Deletion Mutant; or native M-MLV Reverse Transcriptase (RNase H+) was used in a final volume of 25µl. **Reactions** were incubated at 42°C for 60 minutes. TCA precipitations were performed, and first-strand cDNA yields were calculated. Thermostable Polymerases and Reverse Transcriptase Products GoTaq® G2 DNA polymerase is a full-length, recombinant Taq polymerase supplied with buffers designed for enhanced amplification. Reverse transcripase products include AMV, M-MLV and GoScript--an optimized formulation of M-MLV providing robust, reliable cDNA synthesis from a full range of rare and abundant transcripts. View Taq Products and AccessoriesView Reverse Transcriptases and Accessories Example Protocols Example Endpoint PCR Protocol-GoTaq® G2 DNA Polymerase Materials Required GoTaq® G2 DNA Polymerase and Reaction Buffer (Cat.# M7841) PCR Nucleotide Mix (Cat.# C1141) Nuclease-Free Water (Cat.# P1193) upstream primer downstream primer template DNA mineral oil (optional) In a sterile, nuclease-free **microcentrifuge** tube, combine the following components on ice: Component Volume Final Concentration 5X Green or Colorless GoTaq® Reaction Buffer1 10µl 1X (1.5mM MgCl2)2 PCR Nucleotide Mix, 10mM 1µl 0.2mM each dNTP upstream

primer Xµl 0.1–1.0µM downstream primer Yµl 0.1–1.0µM GoTaq® G2 DNA Polymerase (5u/µl) 0.25µl 1.25u template DNA Zµl <0.5µg/50µl Nuclease-Free Water to 50µl 1Thaw completely, and vortex thoroughly prior to use. 2More MgCl2 can be added to the reaction using 25mM MgCl2 Solution (Cat.# A3511) If using a thermal cycler without a heated lid, overlay the reaction mix with 1–2 **drops** (approximately 50µl) of **mineral oil** to prevent evaporation during thermal cycling. **Centrifuge** the reactions in a microcentrifuge for 5 seconds. Place reactions into a thermal cycler that has been heated to 94–95°C and **begin** PCR.

Example
RT Protocol: First-Strand cDNA Synthesis
The following procedure can be used to convert up to 5µg of total RNA or up to 500ng of poly(A) RNA **into first-strand** cDNA. Materials Required GoScript® ReverseTranscription System (Cat.# M5000) High quality experimental RNA Mix and briefly centrifuge each component before use. Combine the following: Xµl Experimental RNA (up to 5µg/reaction) Primer [Oligo(dT)15 (0.5µg/reaction) and/or Random Primer (0.5µg/reaction) or gene-specific primer (10–20pmol/reaction)] Xµl **Nuclease-Free Water** to a final volume of 5µl Heat in a 70°C heat block for 5 minutes. Immediately chill in **ice** water for at least 5 minutes. Centrifuge 10 seconds in a microcentrifuge.

Store on ice until reverse transcription mix is added. Prepare the reverse transcription reaction mix, 15µl for each cDNA reaction. Combine on ice, in the order listed. 4.0µl GoScript™ 5X Reaction Buffer 1.2–6.4µl MgCl2 (final concentration 1.5–5.0mM)1 1.0µl PCR Nucleotide Mix (final concentration 0.5mM each dNTP)2 20units Recombinant RNasin® Ribonuclease Inhibitor (optional) 1.0µl GoScript™ Reverse Transcriptase Xµl Nuclease-Free Water (to a final volume of 15µl) 1Mg2+ concentration should be optimized to 1.5–5.0mM (MgCl2 provided at 25mM). 2If **isotopic** or nonisotopic incorporation is desired for monitoring first-strand cDNA synthesis, α[32P]-dCTP or **other modified** nucleotides **may** be supplemented into the PCR Nucleotide Mix. Combine 15µl of reverse transcription mix with 5µl of RNA and primer mix. Anneal in a heat block at 25°C for 5 minutes. Extend in a heat block at 42°C for up to one **hour**. Reactions can be stopped at this point for analysis of the cDNA or may be frozen for long-term storage. **Inactivate** Reverse Transcriptase: Before proceeding with qPCR, inactivate the reverse transcriptase in a heat block at 70°C for 15 minutes.
https://www.promega.in/resources/guides/nucleic-acid-analysis/pcr-amplification

The majority of PCR methods rely on thermal cycling. Thermal cycling exposes reactants to repeated cycles of heating **and** cooling to permit different temperature-

dependent reactions – specifically, DNA melting and enzyme-driven DNA replication. PCR employs **two** main reagents – **primers** (which are short single strand DNA fragments known as oligonucleotides that are a complementary sequence to the target DNA region) **and** a DNA polymerase. In the **first** step of PCR, the two strands of the DNA **double helix** are physically separated at a high temperature in a **process** called Nucleic acid denaturation. In the **second** step, the temperature is lowered and the **primers** bind to the complementary sequences of DNA. The **two** DNA strands then **become** templates for DNA polymerase to enzymatically assemble a **new** DNA strand from free nucleotides, the building blocks of DNA. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a **chain** reaction in which the original DNA template is exponentially amplified. Almost all PCR applications employ a heat-**stable** DNA polymerase, such as Taq polymerase, an enzyme **originally** isolated from the thermophilic **bacterium** Thermus aquaticus. If the polymerase used was heat-susceptible, it would denature under the high temperatures of the denaturation step. **Before** the use of Taq polymerase, DNA polymerase had to be **manually** added every cycle, which was a tedious and costly process.

PCR amplifies a specific region of a DNA strand (the DNA target). Most PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp) in length,

although some techniques allow for amplification of fragments up to 40 kbp. The amount of amplified product is **determined by** the available **substrates** in the reaction, which become limiting as the reaction progresses. A **basic** PCR set-up requires **several** components and reagents, including: a DNA template that contains the DNA target region to amplify a DNA polymerase; an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common, as it is **more likely to** remain intact during the high-temperature DNA denaturation process two DNA primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target (DNA polymerase can only bind to and elongate from a double-stranded region of DNA; **without** primers there is **no** double-stranded initiation site at which the polymerase can bind); **specific** primers that are complementary to the DNA target region are selected **beforehand**, and are often **custom**-made in a laboratory **or** purchased from commercial biochemical suppliers deoxynucleoside triphosphates, or dNTPs (sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase **synthesizes** a **new** DNA strand a buffer solution providing a suitable chemical environment for optimum activity and stability of the DNA polymerase bivalent cations, typically magnesium (Mg) or manganese (Mn) ions; Mg2+ is the most common, but Mn2+ can be

used for PCR-mediated DNA mutagenesis, as a higher Mn2+ concentration increases the error rate during DNA synthesis; and monovalent cations, typically potassium (K) **ions.** The reaction is commonly carried out in a volume of 10–200 μL in small reaction tubes (0.2–0.5 mL volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below). Many modern thermal cyclers make use of the Peltier effect, which permits both heating and cooling of the block holding the PCR tubes simply by reversing the electric current. Thin-walled reaction tubes permit favorable thermal conductivity to allow for rapid thermal equilibration. Most thermal cyclers have heated lids to prevent condensation at the top of the reaction tube. Older thermal cyclers lacking a heated lid require a layer of **oil** on top of the reaction mixture or a ball of **wax** inside the tube. Typically, PCR consists of a series of 20–40 repeated temperature changes, called thermal cycles, with each cycle commonly consisting of two or three discrete temperature steps. The cycling is often **preceded** by a single temperature step at a very high temperature (>90 °C (194 °F)), and followed by one hold at the end for final product extension or brief **storage**. The temperatures used and the length of time they are applied in each cycle depend on a **variety** of parameters, including the enzyme used for DNA synthesis, the concentration of bivalent

ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers.

The individual steps common to most PCR methods are as follows: **Initialization**: This step is only required for DNA polymerases that require heat activation by hot-start PCR. It consists of heating the reaction chamber to a temperature of 94–96 °C (201–205 °F), or 98 °C (208 °F) if **extremely** thermostable polymerases are used, which is then held for 1–10 minutes. **Denaturation**: This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules. **Annealing**: In the next step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. **Two** different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand. It is **critical** to determine a **proper** temperature for the annealing step because efficiency and specificity are **strongly affected** by the annealing

temperature. This temperature must be **low** enough to allow for hybridization of the primer to the strand, but **high** enough for the hybridization to be specific, i.e., the primer should bind **only** to a perfectly complementary **part** of the strand, and nowhere else.
If the temperature is **too low**, the primer may bind imperfectly. If it is **too high**, the primer may not bind at all. A typical annealing temperature is about 3–5 °C below the Tm of the primers used. Stable hydrogen bonds between complementary bases are formed only when the primer sequence very closely matches the template sequence. During this step, the polymerase binds to the primer-template hybrid and begins DNA formation.
Extension/elongation: The temperature at this step depends on the DNA polymerase used; the **optimum** activity temperature for the thermostable DNA polymerase of Taq (Thermus aquaticus) polymerase is approximately 75–80 °C (167–176 °F), though a temperature of 72 °C (162 °F) is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that are complementary to the template in the 5'-to-3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxy group at the end of the nascent (elongating) DNA strand. The **precise** time required for elongation **depends** both on the DNA polymerase used and on the length of the DNA target region to amplify. As

a rule of thumb, at their **optimal** temperature, most DNA polymerases polymerize a **thousand** bases per minute. Under **optimal** conditions (i.e., if there are **no** limitations due to limiting substrates or reagents), at each extension/elongation step, the number of DNA target sequences is **doubled**.**???** With each successive cycle, the original template strands **plus** all **newly** generated**??** strands become template strands for the next round of elongation, leading to exponential (geometric) amplification of the specific DNA target region. The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions**???** of copies. **The formula used to calculate the number of DNA copies formed after a given number of cycles is 2n,** where n is the number of cycles. Thus, a reaction set for 30 cycles results in 230, **or** 1,073,741,824, copies of the original double-stranded DNA target region. **Final** elongation: This single step is **optional**, but is performed at a temperature of 70–74 °C (158–165 °F) (the temperature range required for optimal activity of most polymerases used in PCR) for 5–15 minutes after the last PCR cycle to ensure that any remaining **single**-stranded DNA is fully elongated. Final hold: The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an **indefinite** time, and may be employed for **short**-term storage of the PCR products.

To check whether the PCR successfully generated the anticipated DNA target region (**also** sometimes referred to as the amplimer or amplicon), agarose gel electrophoresis may be employed for size **separation** of the PCR products. The size(s) of PCR products is **determined by** comparison with a DNA **ladder**, a **molecular weight marker** which contains DNA fragments of **known??** size run on the gel , alongside the PCR products.

Tucker PCR Stages

As with other chemical reactions, the reaction rate **and** efficiency of PCR are **affected** by limiting factors. Thus, the **entire** PCR process can **further** be divided into **three** stages based on reaction progress: **1.**Exponential amplification: At every cycle, the amount of product is doubled (**assuming** 100% reaction efficiency). After 30 cycles, a **single** copy of DNA can be increased up to 1,000,000,000 (one billion) copies. In a sense, then, the replication of a discrete strand of DNA is being manipulated in a **tube** under **controlled** conditions. The reaction is **very** sensitive: only **minute** quantities of DNA must be present. **2.**Leveling off stage: The reaction **slows** as the DNA polymerase loses activity **and** as consumption of **reagents**, such as dNTPs and primers, **causes** them to become more limited. **3.**Plateau: **No** more product accumulates due to exhaustion of reagents and enzyme.

Optimization: PCR optimization
In practice, PCR can **fail** for various reasons, in part due to its sensitivity to contamination causing amplification of spurious DNA products. Because of this, a number of techniques and procedures have been developed for optimizing PCR conditions. Contamination with extraneous DNA is addressed with lab protocols and procedures that separate pre-PCR mixtures from potential DNA contaminants. This usually involves spatial separation of PCR-setup areas from areas for analysis or purification of PCR products, use of disposable plasticware, and thoroughly cleaning the work surface between reaction setups. Primer-design techniques are important in improving PCR product yield and in avoiding the formation of spurious products, and the usage of alternate buffer components or polymerase enzymes can help with amplification of long or otherwise problematic regions of DNA. **Addition** of reagents, such as **formamide**, in buffer systems **may increase** the specificity and yield of PCR.

Computer simulations of theoretical PCR results. (Electronic PCR) may be performed to assist in primer design.

Applications
Selective DNA isolation PCR allows isolation of DNA fragments from genomic DNA by selective amplification

of a **specific region??** of DNA. This use of PCR augments many ways, such as generating hybridization probes for **Southern** or **Northern** hybridization and DNA cloning, which require **larger** amounts of DNA, representing a specific DNA **region**. PCR supplies these techniques with high amounts of **pure** DNA, enabling analysis of DNA samples **even from very small** amounts of starting material. Other applications of PCR include DNA sequencing to determine **unknown** ,PCR-amplified sequences in which **one** of the amplification primers may be used in **Sanger** sequencing, **isolation** of a DNA sequence to expedite recombinant DNA technologies involving the **insertion** of a DNA sequence into a plasmid, phage, **or** cosmid (depending on size) or the genetic material of another organism. **Bacterial** colonies (such as E. coli) can be rapidly screened by PCR for correct DNA vector constructs. PCR may also be used for **genetic fingerprinting**; a forensic technique used to identify a person **or** organism by comparing experimental DNAs through different PCR-based methods. Some PCR **'fingerprints'** methods have high discriminative power and can be used to identify **genetic** relationships between individuals, such as parent-child or between siblings, and are used in **paternity** testing. This technique may also be used to determine **evolutionary** relationships among organisms when certain **molecular** clocks are used (i.e., the 16S rRNA and recA genes of microorganisms).

((genetic- dna fingerprinting is unscientific /very much manipulable /unreliable))

Amplification and **quantification** of DNA.
See also: Use of DNA in forensic entomology.
Because PCR amplifies the **regions** of DNA that it targets, PCR can be used to analyze extremely small amounts of sample. This is often critical for forensic analysis, when **only** a trace amount of DNA is available as evidence. PCR may also be used in the analysis of **ancient** DNA that is **tens of thousands** of years old. These PCR-based techniques have been successfully used on animals, such as a **forty-thousand-**year-old mammoth, and also on human DNA, in applications ranging from the analysis of **Egyptian** mummies to the identification of a Russian **tsar** and the body of **English** king Richard III.

Quantitative PCR or Real Time PCR (qPCR, **not** to be confused with RT-PCR) methods allow the **estimation** of the amount of a given **sequence** present in a sample—a technique often applied to quantitatively determine levels of gene expression. Quantitative PCR is an established tool for DNA quantification that measures the accumulation of DNA product after **each** round of PCR amplification. qPCR **allows** the quantification and detection of a specific DNA sequence in real time since it measures **concentration** , while the synthesis process is taking place. There are **two** methods for simultaneous

detection and quantification. The **first** method consists of using fluorescent **dyes** that are retained **non**specifically in between the double strands. The **second** method involves probes , that code for **specific** sequences and are fluorescently labeled. Detection of DNA using these methods can **only** be seen **after** the hybridization of probes with its complementary DNA takes place. An interesting technique **combination** is real-time PCR **and** reverse transcription. This sophisticated technique, called RT-qPCR, allows for the quantification of a small quantity of RNA. Through this **combined** technique, mRNA is **converted** to cDNA, which is **further** quantified using qPCR. This technique **lowers** the possibility of error at the end point of PCR, **increasing chances???** for detection of genes associated with genetic diseases such as cancer. Laboratories use RT-qPCR for the **purpose** of sensitively measuring gene regulation.

Medical and diagnostic applications
Prospective **parents** can be tested for being genetic carriers, or their children might be tested for actually being affected by a disease**.?????** DNA samples for prenatal testing can be obtained by amniocentesis, chorionic villus sampling, **or even** by the analysis of **rare** fetal cells circulating in the mother's bloodstream. PCR analysis is also **essential** to **pre**implantation genetic diagnosis, where individual cells of a developing embryo are tested for mutations. PCR can also be used as part of a

sensitive test for tissue **typing**, vital to **organ transplantation.???** As of 2008, there is even a proposal to **replace** the traditional **antibody**-based tests for blood **type** , with PCR-based tests. Many forms of cancer involve alterations to oncogenes**??**. By using PCR-based tests to study these mutations, therapy regimens can sometimes be individually customized**???** to a patient. PCR permits**??? early** diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the **highest**-developed**???** in cancer research and is already being used routinely. PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a **sensitivity** that is at least 10,000 fold **higher** than that of other methods. PCR is very useful**???** in the medical field since it allows for the isolation**???** and amplification of tumor suppressors. Quantitative PCR for example, can be used to quantify and analyze **single** cells, as well as recognize DNA, mRNA and protein confirmations and combinations.

Infectious disease applications

PCR allows for rapid and highly **specific** diagnosis of infectious diseases, including those caused by bacteria or viruses. PCR **also** permits identification of **non**-cultivatable **or** slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses from tissue culture assays and animal models. The **basis** for PCR diagnostic applications in microbiology **is** the detection of

infectious **agents** and the discrimination of non-pathogenic from pathogenic strains by virtue of specific **genes**. Characterization and detection of infectious disease organisms have been **revolutionized??** by PCR in the following ways: The human immunodeficiency virus (or HIV), is a difficult target to find and eradicate. The **earliest** tests for infection , relied on the presence of **antibodies** to the virus circulating in the bloodstream. However, antibodies **don't** appear until many weeks after infection , maternal antibodies mask the infection of a newborn, and therapeutic agents to fight the infection **don't** affect the antibodies. PCR tests have been developed that can detect as little as **one** viral genome among the DNA of over 50,000 host cells. Infections can be detected **earlier**, donated blood can be screened **directly** for the virus, **newborns** can be immediately tested for infection, and the effects of **antiviral** treatments can be quantified. Some disease organisms, such as that for **tuberculosis**, are difficult to sample from patients and **slow** to be grown in the laboratory. PCR-based tests have allowed detection of small numbers of disease organisms (both live or dead), in convenient samples. Detailed genetic analysis can also be used to detect **antibiotic resistance**, allowing immediate and effective therapy. The effects of therapy can also be immediately evaluated. The spread of a disease organism through populations of domestic or **wild** animals can be monitored by PCR testing. In many cases, the appearance of **new** virulent

sub-types can be detected and monitored. The **sub**-types of an organism that were responsible for **earlier** epidemics can also be determined by PCR analysis. **Viral DNA** can be detected by PCR. The **primers** used must be **specific** to the targeted sequences in the DNA **of a** virus, and PCR can be used for diagnostic analyses **or** DNA sequencing of the viral genome. The high **sensitivity** of PCR permits virus detection , **soon after** infection and even **before** the onset of disease. Such **early** detection may give physicians a significant **lead** time in treatment. The amount of virus(**"viral load"**) in a patient can also be quantified by PCR-based DNA quantitation techniques. Diseases such as **pertussis** (or whooping cough) are cause by the **bacteria** Bordetella pertussis. This bacteria is marked by a serious acute respiratory infection that affects various **animals** and humans and has led to the deaths of many young children. The pertussis **toxin** is a protein exotoxin that binds to cell receptors by two dimers and reacts with different cell types such as T lymphocytes which plays a role in **cell immunity**. PCR is an important testing tool that can detect the **sequences** that are **within** the pertussis **toxin gene**. This is **because** PCR has a high sensitivity for the toxin and has demonstrated a rapid turnaround time. PCR is very efficient**???** for diagnosing pertussis when compared to **culture**.

Forensic applications

The development of PCR-based genetic (or DNA) **fingerprinting** protocols has seen widespread application in forensics: In its most discriminating form, genetic fingerprinting **can uniquely discriminate any one person from the entire population of the world.!!????** Minute samples of DNA can be **isolated** from a crime scene, and compared to that from suspects, or from a DNA database of earlier evidence or convicts. **Simpler** versions of these tests are often used to rapidly **rule out** suspects during a criminal investigation. Evidence from **decades-old** crimes can be tested, confirming or exonerating the people originally convicted. Forensic DNA typing has been an **effective??** way of identifying or exonerating criminal suspects due to analysis of evidence discovered at a crime scene. The human genome has many **repetitive** regions that can be found **within** gene sequences or in **non**-coding regions of the genome. Specifically, up to **40**% of human DNA is **repetitive**. There are **two** distinct categories for these **repetitive, non**-coding regions in the genome. The **first** category is called **variable** number tandem repeats (VNTR), which are 10–100 base pairs long and the **second** category is called short tandem repeats (STR) and **these** consist of repeated 2–10 base pair sections. PCR is used to **amplify several** well-known VNTRs and STRs using **primers** that **flank** each of the repetitive regions. The **sizes** of the fragments obtained from any individual for each of the STRs will indicate **which** alleles are present. By

analyzing several STRs for an individual, a **set** of alleles for **each** person will be found , that **statistically?? is likely?? to be unique.** Researchers have **identified??** the complete**???** sequence of the human genome. This sequence can be easily **accessed??** through the NCBI website and is used in many **real-life** applications. For example, the FBI has compiled a **set** of DNA **marker** sites used for identification, and **these** are called the Combined DNA Index System (CODIS) DNA database. Using this database enables **statistical** analysis to be used to determine the **probability** that a DNA sample will match. PCR is a very **powerful??** and significant analytical tool to use for forensic DNA typing because researchers only need a very small amount of the target DNA to be used for analysis. For example, **a single human hair** with attached hair **follicle** has enough DNA to conduct the analysis**????.** Similarly, a few **sperm**, skin samples from under the fingernails, or a small amount of blood can provide enough DNA for conclusive analysis. Less discriminating forms of DNA fingerprinting can help in DNA **paternity** testing, where an individual is matched with their close relatives. DNA from **un**identified human remains can be tested, and compared with that from possible parents, siblings, or children. Similar testing can be used to confirm the **biological** parents of an adopted (or kidnapped) child. The actual biological father of a newborn can also be confirmed**???** (or ruled out). The **PCR AMGX/AMGY**

design has been shown to **not** only facilitating in amplifying DNA sequences from a very minuscule amount of genome. However it can **also** be used for real time **sex** determination from forensic bone samples. This provides us with a powerful and effective way to determine the **sex** of not only **ancient** specimens but also current suspects in crimes.

Research applications
PCR has been applied to many areas of research in molecular genetics: PCR allows rapid production of short pieces of DNA, **even** when **not** more than the sequence of the **two** primers is known. This ability of PCR **augments** many methods, such as generating hybridization probes for **Southern** or **Northern** blot hybridization. PCR supplies these techniques with **large amounts of pure???** DNA, sometimes as a **single** strand, enabling analysis even from **very** small amounts of **starting** material. The task of DNA **sequencing** can also be **assisted** by PCR. Known **segments** of DNA can easily be produced **from** a patient with a genetic disease mutation. **Modifications** to the amplification technique can extract **segments** from a **completely unknown** genome, or can generate just a single strand of an area of interest. PCR has numerous applications to the more **traditional** process of DNA **cloning**. It can extract segments **for** insertion into a vector from a larger genome, which may be **only** available in small quantities. Using a single set of 'vector primers', it

can also analyze or extract fragments that have **already** been inserted into vectors. Some **alterations** to the PCR protocol can generate **mutations** (general or site-directed) of an inserted fragment. Sequence-tagged **sites** is a **process** where PCR is used as an indicator that a **particular** segment of a genome is present in a **particular** clone??.

The **Human Genome Project** found this application vital to mapping the cosmid clones they were sequencing, and to coordinating the results from different laboratories. An **exciting** application of PCR is the phylogenic analysis of DNA from **ancient** sources, such as that found in the recovered bones of **Neanderthals**, from frozen tissues of mammoths, or from the brain of **Egyptian** mummies.

Have been amplified and sequenced.
In some cases the **highly** degraded DNA from these sources might be **re**assembled during the **early** stages of amplification. A **common** application of PCR is the study of **patterns** of gene expression. Tissues (or even individual cells) can be analyzed at different stages to see which genes have become **active**, or which have been **switched** off. This application can also use quantitative PCR to quantitate the **actual??** levels of expression.
The ability of PCR to **simultaneously** amplify **several** loci from individual sperm has greatly enhanced the more traditional task of genetic **mapping** by studying

chromosomal crossovers after **meiosis**.(Meiosis is a process where a single cell divides twice to produce four cells containing half the original amount of genetic information. These cells are our sex cells – sperm in males, eggs in females. During meiosis one cell divides twice to form four daughter cells.) **Rare** crossover events between very close loci have been **directly??** observed by analyzing thousands of individual sperms. Similarly, **unusual** deletions, insertions, translocations, or inversions can be analyzed, all without having to wait (or pay) for the long and laborious processes of fertilization, embryogenesis, etc.

Site-directed mutagenesis: PCR can be used to **create mutant** genes **with mutations chosen by scientists at will.** These mutations can be chosen in order to understand how proteins accomplish their functions, and to change or improve protein function.

Advantages
PCR has a number of advantages. It is fairly simple to understand and to use, and produces results rapidly. The technique is highly sensitive with the potential to produce **millions to billions** of copies of a specific product for sequencing, cloning, and analysis. qRT-PCR shares the **same** advantages as the PCR, with an **added** advantage of quantification of the synthesized product. Therefore, it

has its uses to analyze alterations of gene expression levels in tumors, microbes, or other disease states.
PCR is a very powerful and practical **research** tool. The sequencing of **unknown** etiologies of many diseases are being figured out by the PCR. The technique can help identify the sequence of previously **unknown** viruses related to those already known and thus give us a better understanding of the disease itself. **If** the procedure can be further simplified **and** sensitive non radiometric detection systems can be developed, the PCR will assume a prominent place in the clinical laboratory for years to come.

Limitations

One major limitation of PCR is that **prior information about the target sequence is necessary in order to generate the primers that will allow its selective amplification.** This means that, typically, PCR users **must** know the **precise** sequence(s) upstream of the target region on **each** of the two single-stranded templates in order **to ensure** that the DNA polymerase properly binds to the primer-template hybrids , and subsequently **generates** the **entire** target region during DNA synthesis. Like **all enzymes**, DNA polymerases are also prone to error, which in turn causes mutations in the PCR fragments that are generated. **Another** limitation of PCR is that even the **smallest** amount contaminating DNA can be amplified, **resulting in misleading or ambiguous**

results. To minimize the chance of contamination, investigators should **reserve** separate rooms for reagent preparation, the PCR, and analysis of product. Reagents should be dispensed into single-use aliquots. Pipetters with disposable plungers and extra-long pipette tips should be routinely used.

Variants of PCR

Allele-specific PCR: a diagnostic or cloning technique based on single-nucleotide variations (SNVs not to be confused with SNPs) (single-base differences in a patient). It requires **prior** knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3' ends encompass the SNV (base pair buffer around SNV usually incorporated). PCR amplification under **stringent** conditions is **much less efficient** in the presence of a **mismatch** between template and primer, so successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence. See SNP genotyping for more information.

Assembly PCR or Polymerase Cycling

Assembly (PCA): **artificial** synthesis of long DNA sequences by performing PCR on a pool of long oligonucleotides with short overlapping segments. The oligonucleotides alternate between sense and **antisense** directions, and the overlapping segments **determine** the

order of the PCR fragments, thereby **selectively** producing the final long DNA product.

Asymmetric PCR: preferentially amplifies one DNA strand in a double-stranded DNA template. It is used in sequencing and hybridization probing where amplification of **only one of the two** complementary strands is required. PCR is carried out **as usual, but with a great excess of the primer for** the strand targeted for amplification. Because of the **slow** (arithmetic) amplification **later** in the reaction , **after** the limiting primer has been used up, **extra** cycles of PCR are required. A **recent** modification on this process, known as Linear-After-The-Exponential-PCR (LATE-PCR), uses a **limiting** primer with a **higher** melting temperature (Tm) **than** the excess primer to maintain reaction efficiency as the limiting primer concentration decreases mid-reaction.

Convective PCR: a **pseudo**-isothermal way of performing PCR. **Instead** of repeatedly heating and cooling the PCR mixture, the solution is subjected to a thermal gradient. The resulting thermal instability driven convective flow automatically shuffles the PCR reagents from the hot and cold regions repeatedly enabling PCR. Parameters such as thermal boundary conditions and **geometry** of the PCR enclosure can be optimized to yield robust and rapid PCR by harnessing the emergence of **chaotic** flow fields. Such

convective flow PCR setup **significantly** reduces device power requirement and operation time.

Dial-out PCR: a **highly parallel** method for retrieving accurate DNA molecules for gene synthesis. A complex library of DNA molecules is modified with unique flanking tags **before** massively parallel sequencing. **Tag-directed primers** then enable the retrieval of molecules with desired sequences by PCR.

Digital PCR (dPCR): used to measure the quantity of a target DNA sequence in a DNA sample. The DNA sample is **highly diluted** so that after running **many** PCRs in parallel, some of them do **not** receive a single molecule of the target DNA. The target DNA **concentration** is **calculated using the proportion of negative outcomes.** Hence the name 'digital PCR'.

Helicase-dependent amplification: similar to traditional PCR, but uses a **constant** temperature rather than cycling through denaturation and annealing/extension cycles. DNA helicase, an **enzyme** that **unwinds** DNA, is used **in the place of** thermal denaturation.

Hot start PCR: a technique that **reduces non**-specific amplification **during** the initial set up stages of the PCR. It may be performed **manually** by heating the reaction components to the denaturation temperature (e.g., 95 °C)

before adding the polymerase. **Specialized enzyme systems have been developed that inhibit** the polymerase's activity **at** ambient temperature, either by the binding of an **antibody or** by the presence of covalently bound **inhibitors** that dissociate **only after a high**-temperature activation step.

Hot-start/cold-finish PCR is achieved with new hybrid polymerases that are **inactive** at ambient temperature and are **instantly** activated at elongation temperature.

In silico PCR (digital PCR, virtual PCR, electronic PCR, e-PCR) refers to **computational** tools used to calculate **theoretical** polymerase chain reaction results using a given set of primers (probes) to amplify DNA sequences from a sequenced genome or transcriptome. In silico PCR was proposed as an **educational** tool for molecular biology.

Intersequence-specific PCR (ISSR): a PCR method for DNA **fingerprinting** that amplifies regions between simple sequence repeats to produce a unique fingerprint of amplified fragment lengths.

Inverse PCR: is commonly used to identify the flanking sequences around genomic inserts. It involves a series of

DNA **digestions** and self ligation, **resulting** in known sequences at either end of the **un**known sequence.

Ligation-mediated PCR: uses small DNA linkers ligated to the DNA of interest and **multiple** primers annealing to the DNA linkers; it has been used for DNA sequencing, genome **walking**, and DNA **footprinting**.

Methylation-specific PCR (MSP): developed by Stephen Baylin **and** James G. Herman at the Johns Hopkins School of Medicine, and is used to **detect** methylation of CpG **islands** in genomic DNA. DNA is **first** treated with **sodium bisulfite**, which **converts un**methylated cytosine bases to **uracil**, which is recognized by PCR primers as **thymine**. **Two** PCRs are then carried out on the **modified** DNA, using primer **sets** identical except at any CpG islands within the primer sequences. At these points, **one** primer set recognizes DNA **with** cytosines to amplify methylated DNA, and **one** set recognizes DNA with uracil or thymine to amplify unmethylated DNA.

MSP using qPCR can also be performed to obtain qua**nti**tative rather than qualitative information **about** methylation.

Miniprimer PCR: uses a thermostable polymerase (S-Tbr) that can **extend** from short primers ("smalligos") as short

as 9 or 10 nucleotides. This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.

Multiplex ligation-dependent probe amplification (MLPA): permits amplifying multiple targets with a **single** primer pair, thus avoiding the resolution limitations of multiplex PCR .

Multiplex-PCR: consists of multiple primer sets within a single PCR mixture to produce amplicons of varying **sizes** that are specific to different DNA sequences. By targeting multiple **genes??** at once, additional information may be gained from a single test-run that **otherwise** would require several times the reagents and more time to perform. Annealing temperatures for **each** of the primer sets must be optimized to work **correctly** within a single reaction, and amplicon sizes. That is, their base pair length should be different enough to form distinct bands when visualized by gel electrophoresis.

Nanoparticle-Assisted PCR (nanoPCR): some nanoparticles (NPs) can enhance the efficiency of PCR (thus being called nanoPCR), and some can even **outperform** the original PCR **enhancers**. It was reported that quantum dots (QDs) can **improve** PCR specificity and efficiency. Single-walled carbon nanotubes

(SWCNTs) **and** multi-walled carbon nanotubes (MWCNTs) are efficient in enhancing the amplification of long PCR. **Carbon** nanopowder (CNP) can improve the efficiency of **repeated** PCR and **long** PCR, while **zinc** oxide, **titanium** dioxide and **Ag** NPs were found to increase the PCR yield. Previous data indicated that **non-metallic** NPs retained acceptable amplification fidelity. Given that **many** NPs are capable of **enhancing** PCR efficiency, it is clear that there is **likely** to be great potential for nanoPCR technology improvements and product development.

Nested PCR: increases the specificity of DNA amplification, **by** reducing background due to non-specific amplification of DNA. **Two sets** of primers are used in two successive PCRs. In the **first** reaction, one **pair** of primers is used to generate DNA products, which besides the intended target, may still consist of non-specifically amplified DNA fragments. The product(s) are **then** used in a **second** PCR with a set of primers whose binding sites are completely **or** partially different from and located 3' of each of the primers used in the first reaction. Nested PCR is often **more** successful in specifically amplifying long DNA fragments than conventional PCR, **but** it requires more **detailed** knowledge of the target sequences.

Overlap-extension PCR or **Splicing** by overlap extension (SOEing) : a genetic engineering technique that is used to splice together two or **more** DNA fragments that contain complementary sequences. It is used to **join** DNA pieces containing genes, regulatory sequences, or mutations; the technique enables **creation** of specific and long DNA constructs. It can **also** introduce deletions, insertions or point mutations into a DNA sequence.

PAN-AC: uses isothermal conditions for amplification, and may be used in **living** cells.

quantitative PCR (qPCR): used to measure the quantity of a target sequence (commonly in real-time). It quantitatively measures **starting** amounts of DNA, cDNA, or RNA. quantitative PCR is commonly used to determine **whether** a DNA sequence is present in a sample and the number of its copies in the sample. Quantitative PCR has a very **high** degree of precision**??.** Quantitative PCR methods use fluorescent **dyes, such as** Sybr Green, EvaGreen or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time. It is **also** sometimes abbreviated to RT-PCR (real-time PCR) but this abbreviation should be used **only** for reverse transcription PCR. qPCR is the appropriate contractions for quantitative PCR (real-time PCR).

Reverse Transcription PCR (RT-PCR): for amplifying DNA from RNA. Reverse transcriptase , reverse , transcribes RNA into cDNA, which is **then** amplified by PCR. RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start **and** termination sites. **If** the genomic DNA sequence of a gene is **known**, RT-PCR can be **used** to map the location of exons **and** introns in the gene. The **5' end** of a gene (corresponding to the transcription start site) is typically **identified** by RACE-PCR (Rapid Amplification of cDNA Ends). RNase H-dependent PCR (rhPCR): a modification of PCR that utilizes primers with a 3' extension **block** that can be **removed** by a thermostable RNase HII enzyme. This system reduces primer-dimers and **allows** for multiplexed reactions to be performed with **higher numbers of primers.**

Single Specific Primer-PCR (SSP-PCR): allows the amplification of **double**-stranded DNA **even when** the sequence information is available **at one end only.** This method permits amplification of genes for which **only** a **partial** sequence information is available, and allows **uni**directional genome walking from **known** into **un**known regions of the chromosome.

Solid Phase PCR: encompasses **multiple** meanings, including Polony Amplification (where PCR colonies are

derived in a gel matrix, for example), Bridge PCR (primers are covalently linked to a solid-support surface), conventional Solid Phase PCR (where Asymmetric PCR is applied in the presence of solid support bearing primer with sequence **matching** one of the aqueous primers) and **Enhanced** Solid Phase PCR (where conventional Solid Phase PCR can be **improved by** employing high Tm and nested solid support **primer** with optional application of a **thermal** 'step' to favour solid support priming).

Suicide PCR: typically used in paleogenetics or other studies where **avoiding** false positives and **ensuring** the specificity of the amplified fragment is the **highest** priority. It was originally described in a study to verify the presence of the microbe Yersinia pestis in dental samples obtained from **14th** Century graves of people **supposedly** killed by **plague** during the **medieval Black Death epidemic.** The method **prescribes** the use of **any primer** combination **only once** in a PCR (hence the term "suicide"), which should **never** have been used in any positive control PCR reaction, and the primers should **always** target a genomic region **never** amplified before in the lab using this **or** any other set of primers. This ensures that **no** contaminating DNA from previous PCR reactions is present in the lab, which could otherwise generate **false** positives.

Thermal asymmetric interlaced PCR (TAIL-PCR): for **isolation** of an **un**known sequence flanking a known sequence. **Within** the known sequence, TAIL-PCR uses a **nested pair of primers with** differing annealing temperatures; a **degenerate** primer is used to amplify in the other direction from the **un**known sequence.

Touchdown PCR (Step-down PCR): a **variant** of PCR that aims to **reduce** nonspecific background by gradually lowering the annealing temperature as PCR cycling progresses. The annealing temperature at the initial cycles is usually a few degrees (3–5 °C) **above** the Tm of the primers used, while at the later cycles, it is a few degrees (3–5 °C) **below** the primer Tm. The **higher** temperatures give greater specificity for primer binding, and the **lower** temperatures permit more efficient amplification from the specific products formed during the initial cycles.

Universal Fast Walking: for genome walking and genetic fingerprinting using a **more** specific 'two-sided' PCR than conventional 'one-sided' approaches (using **only** one ‘gene-specific primer’ and one general primer—which can lead to **artefactual** 'noise') by virtue of a mechanism involving **lariat** structure formation.

Streamlined derivatives of UFW are LaNe RAGE (lariat-dependent nested PCR for rapid amplification of genomic DNA ends), 5'RACE LaNe and 3'RACE LaNe.

A **1971** paper in the Journal of Molecular Biology by Kjell Kleppe [no] and co-workers in the laboratory of **H. Gobind Khorana** **first** described a method of using an **enzymatic** assay to replicate a short DNA template with primers in **vitro**. However, this early manifestation of the basic PCR principle did **not** receive much attention at the time and the invention of the polymerase chain reaction in **1983** is generally credited to **Kary Mullis.** When Mullis developed the PCR in 1983, he was working in Emeryville, California for **Cetus** Corporation, one of the first biotechnology companies, where he was responsible for synthesizing short chains of DNA. Mullis has written that he **first conceived** the idea for PCR while cruising along the Pacific Coast Highway one night in his car. He was playing in his mind with a new way of analyzing changes (mutations) in DNA when he realized that he had instead invented a method of amplifying any DNA region through repeated cycles of duplication **driven** by DNA polymerase. In Scientific American, Mullis summarized the procedure: "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat." DNA fingerprinting was

first used for paternity testing in 1988. Mullis was awarded the Nobel Prize in Chemistry in 1993 for his invention, seven years after he and his colleagues at **Cetus** first put his proposal to practice. **Mullis's 1985 paper with R. K. Saiki** and **H. A. Erlich,** "Enzymatic Amplification of β-globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia"—the polymerase chain reaction invention (PCR) – was honored by a Citation for Chemical Breakthrough Award from the Division of History of Chemistry of the American Chemical Society in 2017. Some **controversies** have remained about the intellectual and practical contributions of **other** scientists to Mullis' work, and whether he had been the sole inventor of the PCR principle. At the **core** of the PCR method is the use of a suitable DNA polymerase **able** to withstand the high temperatures of >90 °C (194 °F) required for separation of the two DNA strands in the DNA **double helix** after each replication cycle. The DNA polymerases initially employed for in vitro experiments presaging PCR were **unable** to withstand these high temperatures. So the early procedures for DNA replication were very inefficient and time-consuming, and required large amounts of DNA polymerase and continuous handling throughout the process. The discovery in **1976** of Taq polymerase—a DNA polymerase purified from the thermophilic bacterium, Thermus aquaticus, which naturally lives in hot (50 to 80 °C (122 to 176 °F)) environments such as

hot springs—paved the way for dramatic improvements of the PCR method. The DNA polymerase **isolated** from T. aquaticus is stable at high temperatures remaining **active** even after DNA denaturation, thus **obviating** the need to add new DNA polymerase after each cycle. This allowed an **automated** thermocycler-based process for DNA amplification.

- for examinations of the provenance of the key concepts of molecular biology and its **reductionist** thrust and of the role of **rockefeller** foundation in promoting the new field see -- Yoxen = life as productive force, Yoxen - giving life a new meaning , Abir-am -- discourse of physical power , Kohler - Partners in science , Kay - Molecular vision of life . // **Cetus** corporation ,special report ca 1975 . this unpublished report on the commercial potential of genetic engineering was circulated privately in the second half oof 1975 .

Har Gobind Khorana: His **father** was a clerk in the **British** Indian government. In 1945, a fellowship from the **government** of India/ **british** gave him the opportunity to study abroad. He went to the University of Liverpool where he obtained his doctorate. Khorana spent the next few years doing post-doctorate work, first at the Eidgenössische Technische Hochschule in Zurich,

then at **Cambridge** University with **G. W. Kenner** and **Lord Alexander R. Todd.** It was at **Cambridge** that Khorana developed an interest in proteins **and** nucleic acids. --In 1971, he **and** Kjell Kleppe and others laid the **foundations** for the **later** polymerase chain reaction (duplication of DNA sections with DNA polymerases). ---- **Pontifical** Academy of Sciences, Rome, Italy (1978), Foreign Member of the **Royal** Society, London, England (1978), Foreign Member, **Royal** Society of Edinburgh (1982), Order of San Carlos, Government of Columbia, South America (1986)https://www.mediatheque.lindau-nobel.org/laureates/khorana -- **Member American Philosophical Society -- a freemasonry outfit.**
The Philosophical Society, as it was originally called, was **founded** in 1743 by **Benjamin Franklin**, James Alexander (lawyer), Francis Hopkinson, John Bartram, Philip Syng, Jr. and others as an **offshoot** of an earlier club called the **Junto**. It was founded two years after the University of Pennsylvania and still remains closely tied to that institution.
Early members included George **Washington**, John Adams, Thomas Jefferson, Alexander Hamilton, James McHenry, Thomas Paine, David Rittenhouse, **Nicholas Biddle,** Owen Biddle, Benjamin Rush, James Madison, Michael Hillegas, John Marshall, and John Andrews. .... the society also recruited members from **other** countries, including Alexander von **Humboldt**, the Marquis de **Lafayette**, Baron von Steuben, Tadeusz Kościuszko, and

Princess Dashkova. Francis Hopkinson, one of the signatories of the Declaration of Independence. Charles **Darwin**, Robert Frost, Louis **Pasteur**, Elizabeth Cabot Agassiz, John James **Audubon**, Linus **Pauling**, Margaret **Mead**, Maria Mitchell, and Thomas **Edison** became members of the society.

--- Alexander Robertus Todd, Baron Todd OM PRS FRSE . **President** of the Royal Society from 1975 to 1980 and became a member of the Order of Merit in 1977. In 1981, Todd became a **founding** member of the **World Cultural Council.** In 1937 Baron Todd married Alison Sarah Dale (d.1987), daughter of Nobel Prize winner Sir Henry Dale, who, as Todd did, served as **President** of the Royal Society of London. He was **knighted** as Sir Alexander Todd in 1954 ..

Gobind Khorana and the rise of molecular biology
https://science.mit.edu/gobind-khorana-molecular-biology/
Molecular biology is purely THEORETICAL . mathematics , statistics run the show .

From 1970 until his retirement in 2007, Khorana was the **Alfred P. Sloan** Professor of Biology and Chemistry at the Massachusetts Institute of Technology. The Khorana Program was founded in his honor in 2007 by the University of Wisconsin-Madison, the Government of

India, and the Indo-US Science and Technology Forum, with the mission to build a community of scientists, industrialists, and social entrepreneurs in the United States and India.

Modern methods used to synthesize oligonucleotide PCR primers are **still** based on the principles of Khorana's method to make **defined** sequences of DNA for his experiments. www.dnaftb.org/22/bio-2.html

Har Gobind Khorana: Who was the man who helped unlock the **secret** of DNA? The biochemist responsible for discovering an essential function of our DNA — and for constructing the **first synthetic gene .** ... discovering that the order of nucleotides in DNA determines which amino acids are built. Nucleotides are the **subunits** of DNA or RNA, and consist of bases made of nitrogen. There are **four** types of nucleotides for each DNA, and RNA, an the order in which they are put connected — forming the **double helix** — is important for determining which types of proteins the cells create. Certain Proteins are responsible for basic form and functions.

Har Gobind Khorana deciphered DNA and wrote the **dictionary** for our genetic language .. Our understanding of how **genes** shape us owes much to the work of Har Gobind Khorana, ... **mapped out what's now the central dogma of biology** — that information is stored in DNA, a genetic instruction manual, and then transcribed

into RNA, which in turn is translated into the language of proteins. Khorana did stints in research institutions in Switzerland and Canada before landing at the Institute for Enzyme Research and the University of Wisconsin, Madison. There, he **decoded how cells read** the language of RNA written in structures represented by the letters A, C, U, and G. He did this by using **enzymes** to create sequences of these letters. **Arranging** them into distinct patterns, he and other scientists found that the genetic code comprised 64 three-letter "words," known as **codons**. The words code instructions for arranging amino acids, the basic units of proteins. The sequence "GGT" codes the amino acid glycine, for example, while the "UAA" codon tells cellular machinery to stop adding to a nascent protein. Put **together**, the findings yielded something of a Rosetta Stone for genetics, bridging the divide between molecular instructions and the machinery they build. Khorana **went on to** develop a way to make a synthetic gene and then place the **lab-made gene** in a living bacterium.

Vladimir Perlog .. In 1941, in the midst of World War II, Prelog was invited to lecture in Germany by Richard **Kuhn**. Shortly afterwards, Lavoslav Ružička, whom Prelog asked for help, invited Prelog to visit him on his way to Germany. He and his wife used those invitations to escape to Zürich in Switzerland. With Ružička's help, he gained support from **CIBA** Ltd. and started to work in

the Organic Chemistry Laboratory in the Swiss Federal Institute of Technology .

Prelog is rightly considered the premier stereochemist of the second half of the twentieth century. In 1975 he received the Nobel Prize in Chemistry “for his work on the stereochemistry of organic molecules and reactions.” The word stereochemistry is derived from the Greek στ ρόσ (stereos) meaning “solid” and refers to the three-dimensional chemical properties of molecules.

While the perception of the **three**-dimensional aspect goes back to Louis **Pasteur** (1848), and the concept of a **tetrahedral carbon** atom to **Joseph Achille Le Bel** and **Jacobus Henricus van't Hoff** (1874), even in the early twentieth century molecules were still commonly depicted as if they were planar. Such representations conceal important aspects of the mutual interaction of molecules, such as that of a drug with its biological receptor or that of an enzyme with its substrate. In his study of organic chemistry Prelog encountered **Rudolf Lukes,** an enthusiastic young assistant professor, who engaged him as his research assistant. Lukeš worked on alkaloids, and taught Prelog both the **theoretical** and practical aspects of organic chemistry.

a school-mate of Lukes, Gothard J. Dríza, a young entrepreneur who was setting up a laboratory for the preparation of fine chemicals and simultaneously wanted to carry out a doctoral project. Dríza offered Prelog the job of assisting in the laboratory, with the opportunity to

do some research of his own and the additional duty of supervising Dríza's PhD work (officially under Votocek). Prelog chose the **antimalarial** alkaloid quinine for his study, a subject he continued to pursue later in Zagreb and in Zürich. In 1932 he spent nine difficult months in the Royal Yugoslav **Navy**.

Prelog was again able to make contact with a small but prospering commercial pharmaceutical enterprise, Kaštel, Ltd. (later Pliva). Eugen Ladany, an owner of the firm, decided to expand its scope from fabrication of pills, tablets, and injectables to the manufacture of medicinal products not available locally. one of Prelog's first doctoral students devised an inexpensive synthesis of the just-discovered antibacterial drug **sulfanilamide**. This led to financial success for both the company and Prelog and his laboratory and enabled him to spend several months in the laboratory of a fellow Croatian, Leopold Ruzicka, at the ETH (Federal Polytechnic Institute) in Zürich, Switzerland. Back in Zagreb, Prelog tackled two exciting chemical problems: the synthesis of adamantane and the synthesis of quinine and related alkaloids. Adamantane (C10 H16), isolated from Moravian **petroleum** by Stanislav Landa in 1933 has a melting point of 266 degrees Celsius, unusually high for a compound with only **ten** carbon atoms**???**.

Prelog's work had also come to the attention of **Richard Kuhn,**/ In 1943 he became a consultant for **CIBA**.

Prelog met such luminaries as Robert Woodward (Harvard), Robert Robinson (Oxford), Derek H. R. Barton (London), and Maurice Janot (Gif-sur-Yvette, France). Ruzicka persuaded the ETH president to create a second full professorship in the Organic Chemistry Institute, something **very unusual** then in Europe.

CIBA in **Basel** was working on such compounds and enlisted Prelog's help.

Reasoning on the basis of molecular **models**, Prelog concluded that alcohol would give rise to the known (R)-(-)-atrolactic acid and B to the (S)-(+) enantiomer. This hypothesis - **now called Prelog's Rule.**

In 1960 he was elected to the **governing board** of the large Swiss pharmaceutical company CIBA.

.. memberships in national academies, including the Leopoldina, the **Pontifical** Academy, the National Academy of Sciences (USA), the (**British**) Royal Society, and the American **Philosophical** Society. Although **most of** Prelog's research was **basic,** both his background and his industrial connection kept him in touch with applications, especially in drug development.

Khorana's father, **Ganpat Rai,** was a patwai (village agricultural taxation clerk) who worked for the British Indian government.

Khorana greatly valued the philosophy and work ethics **Prelog** passed on to him during this time.

His stay at Cambridge allowed him to witness some of the

greatest discoveries in science, from sequencing of the first protein-insulin by **Frederick Sanger** to determination of what DNA looks like by **Watson and Crick.**

In his words "In my own scientific development, I was most fortunate in coming **under the influence of** a number of very great scientists: Vladimir **Prelog** made me see the beauty in chemistry, work and effort. Later, in biochemistry, I came under the influence of Fritz **Lipmann**, who was so gifted in integrating ideas, and Arthur **Kornberg**, who taught me stringency in biochemical experimentation. **Association with Francis Crick** during and since work on genetic code has been intellectually stimulating and inspiring. Much later, Efraim **Racker** introduced me to membrane biochemistry." The 1960s have been regarded as the golden era of molecular biology and Khorana's own contribution in the development of this field as an independent discipline is **exceptionally** outstanding**??**. In 1961 he made another **breakthrough** by synthesising coenzyme A, a small molecule of immense biological relevance that participates in over **9** per cent of all the chemical reactions occurring within a living cell. He even devised a method to make **several** copies of DNA, which he termed "Repair Synthesis" that was **later** rediscovered by Kary Mullis and **named** as Polymerase Chain Reaction or PCR. It was during this time that he **deciphered** the

biological **language** of genes and demonstrated to the world **how** genes code for proteins that make life.
Following the discovery of the genetic code his interests radically shifted and since then he had been working on mechanisms governing **conversion of light energy to chemical energy by** proteins bacteriorhodopsin and rhodopsin, a biological pigment of the retina, and had about 400 scientific publications to his name in leading journals. He **mentored** over 300 colleagues from all over the world in his lab and faithfully transferred his excellence**????** to them. His first student, Michael Smith, was a recipient of the 1993 Nobel Prize in Chemistry for devising site-directed mutagenesis, a method of **manipulating** DNA. Apart from Nobel, Khorana had been honored with several other prestigious awards which include the **Merck** Award from the Chemical Institute of Canada, the Dannie-Heinneman Prize, the American Chemical Society Award for Creative Work in Synthetic Organic Chemistry, the **Lasker** Foundation Award for Basic Medical Research, the **Padma** Vibhushan Presidential Award, the Ellis Island Medal of Honor, the National Medal of Science, and the Paul Kayser International Award of Merit in **Retina** Research.

---**Richard Kuhn** .. In 1929 he became Principal of the Institute for Chemistry at the **newly** founded **Kaiser Wilhelm/freemason** Institute for Medical Research (which, since 1950, has been **renamed** the **Max**

Planck/theoretical physicist/ occultist Institute for Medical Research in Heidelberg). By 1937 he also took over the administration of this Institute. He was subsequently awarded the Nobel Prize in Chemistry in 1938 for his "work on carotenoids and vitamins," but **rejected** the prize as **Hitler** had forbidden German citizens to accept it. In a **hand**-written letter, he even described the awarding of the prize to a German as an invitation to violate a decree of the **Führer**. He **received** the award after World War II. Kuhn is also credited with the discovery of the **deadly nerve agent Soman** in 1944. Kuhn **collaborated with high-ranking Nazi officials** and denounced three of his Jewish co-workers in 1936. In 2005, the Society of German Chemists (Gesellschaft Deutscher Chemiker, GDCh) declared their intention to **no longer** award the Richard Kuhn Medal: “The board of the GDCh intends to discontinue awarding the Medal named after the organic chemist, Nobel Prize laureate of the year 1938 and President of the GDCh in 1964–65, Richard Kuhn. The board thereby draws the consequences out of research on Richard Kuhn’s behaviour during National Socialism. Even though the question of whether Kuhn was a convinced National Socialist or just a career-oriented camp follower is not fully answered, he undisputably supported the **Nazi**-regime in administrative and organizational ways, especially by his scientific work. Despite his scientific achievements, **Kuhn is not suitable to serve as a role model,** and eponym for an important

award, mainly due to his unreflected research on poison gas, but also due to his conduct towards Jewish colleagues" (Nachrichten aus der Chemie 54, May 2006, p. 514). ///// Second World War and invention of poison gas . As an Austrian, Kuhn did not have to join the NSDAP but – according to the current state of research – compensated for this with his all-German convictions and adaptation to the regime. In 1933 he dismissed his Jewish members of staff. In order to secure resources for his own research and further his career, he also denounced a colleague who continued to employ Jewish workers. When the war began, Kuhn investigated means of protection against chemical warfare. Conversely, from 1940 he conducted research into vitamin **inhibitors** on behalf of the Wehrmacht with regard to their use as chemical weapons. At the end of 1942, Kuhn additionally turned his attention to **poison** gas research. Initially, he sought defence substances to protect one's own troops. After the supposed antidote actually augmented the effect of the novel nerve gases **Tabun** and Sarin even further, however, he developed the poison gas **Soman**, which was – and still is to this day – far more lethal than the other two due to the lack of medical treatment possibilities. As a member of the war research network, he was also **indirectly** involved in experiments **on humans.** Cooperation with the Allies …. In autumn 1944, files on Soman research were **removed** from Heidelberg by chemical officers, documents and letters on other projects

destroyed. For lack of concrete documentation, Kuhn was able to convince the Western Allies that he had actually prevented worse in his various functions within war research. His willingness to cooperate with the Western powers and their interest in his biochemical knowhow enabled him to continue on as Head of the Kaiser Wilhelm Institute for Medical Research (which would eventually be renamed the Max Planck Institute for Medical Research). From 1948 he was Secretary of the Supervisory Board, from 1954 Chairman of the Chemistry, Physics and Technology Section and from 1955 Vice-President of the Kaiser Wilhelm/Max Planck Society. // Letter from Richard Kuhn to the President of ETH Zurich, Munich, 10 July 1926, accepting his appointment to the Chair of General Chemistry at ETH Zurich (ETH-Bibliothek, University Archives, SR3: 1926/No.1034). Holdings ETH Zurich's University Archives at ETH-Bibliothek contain the Historical School Board Archive with documentation on Richard Kuhn's chair. A correspondence spanning thirty-four letters between Richard Kuhn **and** Arthur Stoll (1887 to 1971), a professor of chemistry at the University of Munich and Director of **Sandoz AG Basel,** from the years 1932 to 1957 is archived in the combined personal papers of Richard Willstätter and Arthur Stoll.

Patent disputes The PCR technique was **patented** by Kary Mullis and assigned to Cetus Corporation, where Mullis worked when he invented the technique in 1983. The Taq polymerase enzyme was **also** covered by patents. There have been several high-profile **lawsuits** related to the technique, including an unsuccessful lawsuit brought by **DuPont**. The Swiss pharmaceutical company Hoffmann-La **Roche/ rothschilds** purchased the rights to the patents in 1992 and currently holds those that are **still** protected. A related patent **battle** over the Taq polymerase enzyme is still ongoing in several jurisdictions around the world between Roche and Promega. The legal arguments have extended beyond the lives of the original PCR and Taq polymerase patents, which expired on March 28, 2005.

https://en.wikipedia.org/wiki/History_of_polymerase_chain_reaction

Loop-mediated isothermal amplification (LAMP) is a single-tube technique for the amplification of DNA and a low-cost alternative to detect certain diseases. Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) **combines** LAMP with a reverse transcription step to allow the detection of RNA. LAMP is an isothermal nucleic acid amplification technique. In contrast to the polymerase chain reaction (PCR) technology, in which the reaction is carried out with a

series of alternating temperature steps or cycles, **isothermal** amplification is carried out at a **constant** temperature, and does **not** require a thermal cycler. In LAMP, the target sequence is amplified at a constant temperature of 60–65 °C using either two or three sets of primers and a polymerase with high strand displacement activity in addition to a replication activity. Typically, **4 different primers** are used to amplify **6 distinct regions** on the target gene, which **increases** specificity. An **additional** pair of "loop primers" can further accelerate the reaction. The amount of DNA produced in LAMP is considerably **higher** than PCR-based amplification. The amplification product can be detected via photometry, measuring the turbidity **caused** by **magnesium** pyrophosphate precipitate in solution as a **byproduct** of amplification. This allows easy **visualization** by the naked eye **or via** simple photometric detection approaches for small volumes. The reaction can be followed in real-time either by measuring the turbidity or by fluorescence using intercalating **dyes** such as SYTO 9. Dyes, such as SYBR green, can be used to create a **visible** color change that can be seen with the naked eye without the need for expensive equipment, or for a response that can more accurately be measured by instrumentation. Dye molecules intercalate or directly label the DNA, and in turn can be correlated with the number of copies initially present. Hence, LAMP can **also** be quantitative. In-tube detection of LAMP DNA amplification is possible using

manganese loaded **calcein** which starts fluorescing upon complexation of manganese by pyrophosphate during in vitro DNA synthesis. **Another** method for visual detection of the LAMP amplicons by the unaided eye was **based** on their ability to hybridize with complementary gold-bound ss-DNA and **thus** prevent the normal red to purple-blue color change that would otherwise occur during salt-induced aggregation of the gold particles. So, a LAMP method combined with amplicon detection by AuNP can have advantages over other methods in terms of reduced assay time, amplicon confirmation by hybridization and use of simpler equipment (i.e.,**no need** for a thermocycler, electrophoresis equipment or a UV trans-illuminator.

LAMP is a **relatively** new DNA amplification technique, which due to its simplicity, ruggedness, and low cost could provide major advantages. LAMP has the potential to be used as a simple screening assay in the field or at the point of care by clinicians. Because LAMP is isothermal, which eradicates the need for expensive thermocyclers used in conventional PCR, it may be a particularly useful method for **infectious** disease diagnosis **in low and middle income countries.** LAMP is widely being studied for detecting **infectious** diseases such as tuberculosis, malaria, and sleeping sickness. In **developing** regions, it has **yet to be** extensively validated for other common pathogens. LAMP has been observed to be **less sensitive** (more resistant) than PCR to inhibitors

in complex samples such as **blood**, **likely** due to use of a different DNA polymerase (typically Bst – Bacillus stearothermophilus – DNA polymerase rather than Taq polymerase as in PCR). Several reports describe successful detection of pathogens from minimally processed samples such as **heat-treated blood,** or in presence of **clinical** sample matrices. This feature of LAMP may be **useful** in low-resource or field settings where a conventional DNA or RNA extraction prior to diagnostic testing may be impractical.

Limitations

LAMP is less versatile than PCR, the most familiar nucleic acid amplification technique. LAMP is useful primarily as a **diagnostic** or detection technique, but is not useful for cloning or many other molecular biology applications enabled by PCR. Because LAMP uses **4 (or 6) primers** targeting 6 (or 8) regions within a fairly small segment of the genome, **and because primer design is subject to numerous constraints, it is difficult to design** primer sets for LAMP "by eye". Free, open-source or commercial **software** packages are generally used to assist with LAMP primer design, although the primer design constraints mean there is less freedom to choose the target site than with PCR. In a **diagnostic** application, this must be balanced against the need to choose an appropriate target (e.g., a conserved site in a highly

variable viral genome, or a target that is specific for a particular strain of pathogen). Multiple degenerated sequences may be required to cover the different variant strains of the same species. A consequence of having such a **cocktail** of primers can be **non**-specific amplification in the late amplification. Multiplexing approaches for LAMP are **less** developed than for PCR. The larger number of **primers per target** in LAMP increases the likelihood of primer-primer **interactions** for multiplexed target sets. The product of LAMP is a series of concatemers of the target region, giving rise to a characteristic "ladder" or banding pattern on a gel, rather than a single band as with PCR. Although this is not a problem when detecting single targets with LAMP, "traditional" (endpoint) multiplex PCR applications wherein identity of a target is **confirmed** by size of a band on a gel are not feasible with LAMP. Multiplexing in LAMP has been achieved by choosing a target region with a restriction site, and digesting prior to running on a gel, such that each product gives rise to a distinct size of fragment, although this approach adds **complexity** to the experimental design and protocol. The use of a strand-displacing DNA polymerase in LAMP also **precludes** the use of hydrolysis probes, e.g. TaqMan probes, which rely upon the 5'-3' exonuclease activity of Taq polymerase. An alternative real-time multiplexing approach based on fluorescence quenchers has been reported. SYBR green dye may be added to view LAMP in real-time. However,

in the late amplification, primer-dimer amplification may contribute to a false positive signal. Unlike traditional SYBR-green-based PCR assays, a melt curve analysis cannot be performed in LAMP to check for the presence of primer dimers.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a technique for the amplification of RNA. Within the last 10 years of its development, applications of the LAMP method in pathogenic microorganisms, genetically modified ingredients, tumor detection, and embryo sex identification have been widely used. This method was then improved by taking it a step further and combining it with a reverse transcription phase to allow for the detection of RNA. RT-LAMP is a one step nucleic acid amplification method that is used to diagnose **infectious** disease caused by **bacteria** or viruses. Although it has **not** been formally recognised by NAT, the method has been developed into many **commercial** kits that can be used for the identification of pathogens. The commonly used PCR method is able to generate millions of copies of the target strand. This process relies on thermal cycling, cycles of heating **and** cooling to facilitate the DNA replication. RT-LAMP does **not** require these cycles and is performed at a **constant** temperature between 60 and 65 °C. **Similar** to RT-PCR, RT-LAMP uses reverse transcriptase to synthesize complementary DNA (cDNA)

from RNA sequences. This cDNA is **then** amplified using DNA polymerase, generating 10^9 copies per hour.
RT-LAMP is used in the detection of **viruses**. This method can be very effective in detecting **viruses** with an RNA genome (Group II, IV, and V based on the Baltimore Virus Classification system). **Four** specially designed **primers** recognize distinct target sequences on the template strand. The primers bind **only** to these sequences which allows for high specificity. Out of the 4 primers involved, two of them are "**inner** primers" (FIP and BIP) which are designed to synthesize **new** DNA strands. The outer primers (F3 and B3) anneal to the template strand and also generate **new** DNA. These primers are accompanied by DNA polymerase which aids in strand displacement and releases the **newly** formed DNA strands. The BIP primer, accompanied by reverse transcriptase, initiates the process by binding to a target sequence on the 3' end of the RNA template and synthesizing a copy DNA strand. The B3 primer binds to this side of the template strand as well, and with the help of DNA polymerase simultaneously **creates** a new cDNA strand while **displacing** the previously made copy. The double stranded DNA containing the template strand is **no** longer needed. The single stranded copy now loops at the 3' end as it binds to itself. The FIP primer binds to the 5' end of this single strand and accompanied by DNA polymerase, synthesizes a complementary strand. The F3 primer, with DNA polymerase, binds to this end and

generates a new double stranded DNA molecule while displacing the previously made single strand. This new single strand that has been released will act as the starting point for the LAMP cycling amplification. The DNA has a dumbbell-like structure as the ends fold in and self anneal. This structure becomes a stem-loop when the FIP or BIP primer once again initiates DNA synthesis at one of the target sequence locations. This cycle can be started from either the forward **or** backward side of the strand using the appropriate primer. Once this cycle has begun, the strand undergoes **self**-primed DNA synthesis during the elongation stage of the amplification process. This amplification takes place in only an hour, under isothermal conditions between 60-65 °C.

This method is specifically advantageous because it can all be done quickly in one step. The sample is mixed with the primers, reverse transcriptase and DNA polymerase and the reaction takes place under a constant temperature. The required temperature is so low that the reaction can be completed using a simple hot water bath. There is no need for expensive thermocycling equipment that is necessary for other methods like PCR, which makes RT-LAMP very cost effective. In contrast with conventional PCR and real-time PCR assays, this method is much more **efficient** while still obtaining a **high** level of precision. This inexpensive and streamlined method can be more readily used in **developing** countries that do **not** have

access to high tech laboratories. These areas are known for having a multitude of infectious diseases caused by various bacteria and viruses**???**. The LAMP method is very useful for detection of these pathogens.

A **disadvantage** of this method is generating the sequence specific primers. For each LAMP assay, primers must be **specifically** designed to be compatible with the target DNA. This can be **difficult** which **discourages** researchers from using the LAMP method in their work. There is however, a free **software** called Primer Explorer, developed by Fujitsu in Japan, which can aid in the selection of these primers.

Viruses infect host cells with their specific genetic information, which the cell then replicates, causing the host to become diseased. In an effort to identify which certain virus is present in a host, RT-LAMP is used to test for the specific sequence of the virus, **made possible by comparing the sequences against a large external database of references.??? ((how the database is created???))** A primary example of the RT-LAMP was as an experiment to detect a new duck Tembusu-like, BYD virus, named after the region, Baiyangdian, where it was first isolated**??**. Because the symptoms of this virus were **similar** to Tembusu, an **already** identified disease, the nucleotide sequence of the complete genome of this virus was available in **external** resources**???**. The **known**

sequence was put into the online primer-designing software, LAMP Primer Explorer (http://primerexplorer.jp/e/), where the appropriate primers were designed **and** selected. With the selected primers, a RT-LAMP assay was done to amplify the RNA, with which the samples could then be visualized and confirmed under natural **and** UV light. Another recent application of this method, was in a 2013 experiment to detect an Akabane virus using RT-LAMP. The experiment, done in **China**, isolated the virus from aborted calf fetuses, which is rarely successful but was able to be done because of RT-LAMP's easy detection feature and high sensitivity. With the use of the Primer Explore V4 Software, and a sequence reference of the Akabane virus, the correct primers were developed and tested in an RT-LAMP assay. For specification purposes, the assay was also run against 4 **other** virus **known??** to cause **abortion** in cattle. These comparative assays were **un**successful due to the primers not binding to the template regions.

DNA **spiking**, **also** known as custom spiking, is the differing ratio of bases at a single degenerate position when synthesizing oligonucleotides. DNA spiking can include either equal or unequal proportions of bases at a given position (for example, 10% Adenine, 75% Guanine, 5% Cytosine & 10% Thymine). As an example, with the

degenerate code R = A + G, 50% of the time that R position is adenine and the other 50% of the time it is guanine. However, with DNA Spiking, the R position could be adenine 70% of the time and guanine 30% of the time. The proportions do **not** need to be 70:30, the ratios can be anything else such as 12:82 and 64:36.
DNA spiking can **also** refer to a spike control in PCR, which is when DNA is **added** to a sample that will provide some signal (e.g. a plasmid or some synthetic DNA with a specific **known** sequence) to a reaction, and seeing if**??** the reaction will amplify. This method is used to discover **if** the PCR method is working correctly, as in a PCR machine it may **not** amplify DNA properly, so by adding **spiked** DNA it can be observed how much DNA is produced. This is then compared to the amount of DNA that would be **theoretically** predicted if the machine was working properly so that any malfunctions can be discovered.

The **selector** technique is a method to amplify and multiplex genomic DNA. Genomic DNA is **digested** with restriction enzymes, circularized by hybridisation to selectors and subsequently attached to a vector sequence by ligation. The procedure **results** in circular DNA molecules with an included general primer pair motif that can be used for amplification by PCR or RCA.

A selector consists of two oligonucleotides, one Vector oligonucleotide and one Selector probe. Together they form one Selector with target specific ends on each side of a general primer motif. Selection mechanisms A selector probe hybridizes with both ends of the selected target. A selector probe hybridizes with one end to the 3’ end of the target and the other end to an internal sequence of the target. The protruding 5' end is cleaved off using Taq polymerase.

DNA polymerase is an **enzyme** that synthesizes DNA molecules from deoxyribonucleotides, the building blocks of DNA. These enzymes are **essential** for DNA replication and usually work in **pairs** to create two identical DNA strands **from** a single original DNA molecule. During this process, DNA polymerase "reads" the existing DNA strands **to create** two new strands that match the existing ones. These enzymes catalyze the chemical reaction deoxynucleoside triphosphate + DNAn $\rightleftharpoons$ diphosphate + DNAn+1. DNA polymerase adds nucleotides to the three prime (3')-end of a DNA strand, one nucleotide at a time. Every time a cell **divides**, DNA polymerases are **required** to help duplicate the cell's DNA, so that a copy of the original DNA molecule can be passed to each daughter cell. In this way, genetic information is passed down **from** generation to generation. **Before** replication can take place, an **enzyme**

called **helicase** unwinds the DNA molecule from its tightly woven form, in the process **breaking** the hydrogen bonds between the nucleotide bases. This opens up or "unzips" the double-stranded DNA to give two single strands of DNA that can be used as templates for replication. In **1956**, **Arthur Kornberg** and colleagues discovered DNA polymerase I (Pol I), in Escherichia coli. They described the DNA replication **process** by which DNA polymerase copies the base sequence of a template DNA strand. Kornberg was later awarded the Nobel Prize in Physiology or Medicine in 1959 for this work. DNA polymerase II was **also** discovered by Thomas Kornberg (the son of Arthur Kornberg) and Malcolm E. Gefter in 1970 while further elucidating the role of Pol I in E. coli DNA replication. The **main** function of DNA polymerase is to **synthesize** DNA from deoxyribonucleotides, the building blocks of DNA. The DNA copies are **created** by the pairing of nucleotides to bases present on each strand of the original DNA molecule. This **pairing** always occurs in specific combinations, with cytosine along with guanine, and thymine along with adenine, forming two separate pairs, respectively. By **contrast**, RNA polymerases synthesize RNA from ribonucleotides from either RNA or DNA. When synthesizing new DNA, DNA polymerase can add free nucleotides **only** to the 3' end of the newly forming strand. This **results** in elongation of the newly forming strand in a 5'–3' direction. **No known DNA polymerase**

is able to begin a new chain (de novo); it can only add a nucleotide onto a **pre**-existing 3'-OH group, and therefore needs a primer at which it can add the first nucleotide. Primers consist of RNA or DNA bases (or both). In DNA replication, the **first** two bases are always RNA, and are synthesized by **another** enzyme called primase. Helicase and topoisomerase II are required to **unwind** DNA from a double-strand structure to a single-strand structure to facilitate replication of each strand consistent with the semiconservative model of DNA replication. It is important to note that the directionality of the newly forming strand (the daughter strand) is **opposite** to the direction in which DNA polymerase moves along the template strand. Since DNA polymerase requires a free 3' OH group for initiation of synthesis, it can synthesize in only one direction by extending the 3' end of the preexisting nucleotide chain. Hence, DNA polymerase moves along the template strand in a 3'–5' direction, and the daughter strand is formed in a 5'–3' direction. This difference enables the resultant double-strand DNA formed to be composed of two DNA strands that are antiparallel to each other. The function of DNA polymerase is **not** quite perfect, with the enzyme making about **one** mistake for every **billion** base pairs copied**???.** Error correction is a property of some, but not all DNA polymerases. This process corrects mistakes in newly synthesized DNA. When an incorrect base pair is recognized, DNA polymerase moves **backwards** by one

base pair of DNA. The 3'–5' exonuclease activity of the enzyme allows the incorrect base pair to be excised (this activity is known as proofreading). Following base excision, the polymerase can **re**-insert the correct base and replication can continue forwards. This preserves the integrity of the original DNA strand that is passed onto the daughter cells. **Fidelity** is very important in DNA replication. **Mismatches** in DNA base pairing can potentially **result** in dysfunctional proteins and could lead to cancer. Many DNA polymerases contain an exonuclease domain, which acts in detecting base pair mismatches and further performs in the removal of the incorrect nucleotide to be replaced by the correct one.
The shape and the interactions accommodating the **Watson** and **Crick** base pair are what primarily contribute to the detection or error. **Hydrogen** bonds play a key role in base pair binding and interaction. The loss of an interaction, which occurs at a mismatch, is said to trigger a shift in the balance, for the binding of the template-primer, from the polymerase, to the exonuclease domain. In addition, an incorporation of a **wrong** nucleotide causes a **retard** in DNA polymerization. This delay gives time for the DNA to be **switched** from the polymerase site to the exonuclease site. Different conformational changes and loss of interaction occur at different mismatches. In a purine: pyrimidine mismatch there is a displacement of the pyrimidine towards the major groove and the purine towards the minor groove.

Relative to the shape of DNA polymerase's binding pocket, steric clashes occur between the purine and residues in the minor groove, and important van der Waals and electrostatic interactions are lost by the pyrimidine. Pyrimidine:pyrimidine and purine:purine mismatches present less notable changes since the bases are displaced towards the major groove, and less steric hindrance is experienced. However, although the different mismatches result in different steric properties, DNA polymerase is still able to detect and differentiate them so uniformly and maintain fidelity in DNA replication. DNA polymerization is also **critical** for many mutagenesis processes and is widely employed in biotechnologies.

The known DNA polymerases have **highly** conserved structure, which means that their overall catalytic subunits vary very little from species to species, independent of their domain structures. Conserved structures usually indicate important, irreplaceable functions of the cell, the maintenance of which provides evolutionary advantages. The shape can be described as resembling a right hand with thumb, finger, and palm domains. The palm domain appears to function in catalyzing the transfer of phosphoryl groups in the phosphoryl transfer reaction. DNA is bound to the palm when the enzyme is active. This reaction is believed to be catalyzed by a two-metal-ion mechanism. The finger domain functions to bind the nucleoside triphosphates with the template base. The

thumb domain plays a potential role in the processivity, translocation, and positioning of the DNA.

DNA polymerase's rapid catalysis is due to its processive nature. Processivity is a **characteristic** of enzymes that function on polymeric substrates. In the case of DNA polymerase, the degree of processivity refers to the average number of nucleotides added each time the enzyme binds a template. The average DNA polymerase requires about one second locating and binding a primer/template junction. Once it is bound, a nonprocessive DNA polymerase adds nucleotides at a rate of one nucleotide per second.

Processive DNA polymerases, however, add multiple nucleotides per second, drastically increasing the rate of DNA synthesis. The degree of processivity is directly proportional to the rate of DNA synthesis. The rate of DNA synthesis in a living cell was first determined as the rate of phage T4 DNA elongation in phage infected E. coli. During the period of exponential DNA increase at 37 °C, the **rate was 749 nucleotides per second.** DNA polymerase's ability to slide along the DNA template allows increased processivity. There is a **dramatic** increase in processivity at the replication fork. This increase is facilitated by the DNA polymerase's association with proteins known as the sliding DNA **clamp**. The clamps are multiple protein subunits associated in the **shape** of a ring. Using the hydrolysis of

ATP, a class of proteins known as the sliding clamp loading proteins open up the ring structure of the sliding DNA clamps allowing binding to and release from the DNA strand. Protein-protein interaction with the clamp prevents DNA polymerase from diffusing from the DNA template, thereby ensuring that the enzyme binds the same primer/template junction and continues replication. DNA polymerase changes conformation, increasing affinity to the clamp when associated with it and decreasing affinity when it completes the replication of a stretch of DNA to allow release from the clamp.
Based on sequence homology, DNA polymerases can be further subdivided into **seven** different families: A, B, C, D, X, Y, and RT. Some **viruses** also encode special DNA polymerases, such as Hepatitis B virus DNA polymerase. These may selectively replicate viral DNA through a variety of mechanisms. **Retroviruses** encode an **unusual** DNA polymerase called **reverse** transcriptase, which is an RNA-dependent DNA polymerase (RdDp). It polymerizes DNA from a template of RNA.

Prokaryotic polymerases exist in two forms: core polymerase and holoenzyme. Core polymerase synthesizes DNA from the DNA template but it **cannot** initiate the synthesis alone or accurately. Holoenzyme accurately initiates synthesis. Pol I Prokaryotic family A polymerases include the DNA polymerase I (Pol I) enzyme, which is encoded by the polA gene and

ubiquitous among prokaryotes. This repair polymerase is involved in excision repair with both 3'–5' and 5'–3' exonuclease activity and processing of Okazaki fragments generated during lagging strand synthesis. Pol I is the most abundant polymerase, accounting for >95% of polymerase activity in E. coli; yet cells lacking Pol I have been found suggesting Pol I activity can be replaced by the other four polymerases. Pol I adds ~15-20 nucleotides per second, thus showing poor processivity. Instead, Pol I starts adding nucleotides at the RNA primer:template junction known as the **origin** of replication (ori). Approximately 400 bp downstream from the origin, the Pol III holoenzyme is assembled and takes over replication at a highly processive speed and nature.

Taq polymerase is a heat-stable enzyme of this family that **lacks** proofreading ability. Pol II DNA polymerase II is a family B polymerase encoded by the polB gene. Pol II has 3'–5' exonuclease activity and participates in DNA repair, replication restart to bypass lesions, and its cell presence can jump from ~30-50 copies per cell to ~200–300 during SOS induction. Pol II is also **thought to be** a backup to Pol III as it can interact with holoenzyme proteins and assume a high level of processivity. The main role of Pol II is **thought to be** the ability to direct polymerase activity at the replication fork and helped stalled Pol III bypass terminal mismatches. Pfu DNA polymerase is a heat-stable enzyme of this family found in the hyperthermophilic archaeon Pyrococcus furiosus.

Detailed classification divides family B in archaea into B1, B2, B3, in which B2 is a group of pseudoenzymes. Pfu belongs to family B3. Others PolBs found in archaea are part of "Casposons", Cas1-dependent transposons. Some viruses (including Φ29 DNA polymerase) and mitochondrial plasmids carry polB as well. Pol III DNA polymerase III holoenzyme is the **primary** enzyme involved in DNA replication in E. coli **and** belongs to family C polymerases. It consists of three assemblies: the pol III core, the beta sliding clamp processivity factor, and the clamp-loading complex. The **core** consists of three subunits: α, the polymerase activity hub, ε, exonucleolytic proofreader, and θ, which may act as a stabilizer for ε. The beta sliding clamp processivity factor is also present in duplicate, one for each core, to create a clamp that encloses DNA allowing for high processivity. The third assembly is a **seven**-subunit (τ2γδδ′χψ) clamp loader complex. Recent research has classified Family C polymerases as a subcategory of Family X with no eukaryotic equivalents. The old textbook "trombone model" depicts an elongation complex with two equivalents of the core enzyme at each replication fork (RF), one for each strand, the lagging and leading. However, recent evidence from single-molecule studies indicates an average of three stoichiometric equivalents of core enzyme at each RF for both Pol III and its counterpart in B. subtilis, PolC. In-cell fluorescent microscopy has revealed that leading strand synthesis

may not be completely continuous, and Pol III* (i.e., the holoenzyme α, ε, τ, δ and χ subunits without the ß2 sliding clamp) has a high frequency of dissociation from active RFs. In these studies, the replication fork turnover rate was about 10s for Pol III*, 47s for the ß2 sliding clamp, and 15m for the DnaB helicase. This suggests that the DnaB helicase may remain stably associated at RFs and serve as a nucleation point for the competent holoenzyme. In vitro single-molecule studies have shown that Pol III* has a high rate of RF turnover when in excess, but remains stably associated with replication forks when concentration is limiting. Another single-molecule study showed that DnaB helicase activity and strand elongation can proceed with decoupled, stochastic kinetics. Pol IV In E. coli, DNA polymerase IV (Pol IV) is an **error**-prone DNA polymerase involved in non-targeted mutagenesis. Pol IV is a Family Y polymerase expressed by the dinB gene that is switched on via SOS induction caused by stalled polymerases at the replication fork. During SOS induction, Pol IV production is increased **tenfold** and one of the functions during this time is to interfere with Pol III holoenzyme processivity. This creates a checkpoint, stops replication, and allows time to **repair** DNA lesions via the appropriate repair pathway. Another function of Pol IV is to perform translesion synthesis at the stalled replication fork like, for example, bypassing N2-deoxyguanine adducts at a **faster** rate than transversing undamaged DNA. Cells

lacking dinB gene have a higher rate of mutagenesis caused by DNA damaging agents. Pol V DNA polymerase V (Pol V) is a Y-family DNA polymerase that is involved in SOS response and translesion synthesis DNA repair mechanisms. Transcription of Pol V via the umuDC genes is highly regulated to produce only Pol V when damaged DNA is present in the cell generating an SOS response. Stalled polymerases **causes** RecA to bind to the ssDNA, which **causes** the LexA protein to autodigest. LexA then **loses** its ability to repress the transcription of the umuDC operon. The same RecA-ssDNA nucleoprotein posttranslationally modifies the UmuD protein into UmuD' protein. UmuD and UmuD' form a heterodimer that interacts with UmuC, which in turn activates umuC's polymerase catalytic activity on damaged DNA. In E. coli, a polymerase “tool belt” model for switching pol III with pol IV at a stalled replication fork, where both polymerases bind simultaneously to the β-clamp, has been proposed. However, the involvement of more than one TLS polymerase working in succession to bypass a lesion has **not** yet been shown in E. coli. Moreover, Pol IV can catalyze both insertion and extension with high efficiency, whereas pol V is considered the major SOS TLS polymerase. One example is the bypass of intra strand guanine thymine cross-link where it was shown on the basis of the difference in the mutational signatures of the two polymerases, that pol IV and pol V compete for TLS of the intra-strand crosslink.

In 1998,the family D of DNA polymerase was **discovered** in Pyrococcus furiosus and Methanococcus jannaschii. The PolD complex is a heterodimer of two chains, each encoded by DP1 (small proofreading) and DP2 (large catalytic). Unlike other DNA polymerases, the structure and mechanism of the catalytic core **resemble** that of multi-subunit RNA polymerases. The DP1-DP2 interface resembles that of Eukaryotic Class B polymerase zinc finger and its small subunit. DP1, a Mre11-like exonuclease, is **likely** the precursor of small subunit of Pol α and ε, providing proofreading capabilities now **lost** in Eukaryotes. Its N-terminal HSH domain is similar to AAA proteins, especially Pol III subunit δ and RuvB, in structure. DP2 has a Class II KH domain. Pyrococcus abyssi polD is **more heat-stable and more accurate than Taq polymerase, but has not yet been commercialized.** Eukaryotic DNA polymerase Polymerases β, λ, σ and μ (beta, lambda, sigma, and mu) Family X polymerases contain the well-known eukaryotic polymerase pol β (beta), as well as other eukaryotic polymerases such as Pol σ (sigma), Pol λ (lambda), Pol μ (mu), and Terminal deoxynucleotidyl transferase (TdT). Family X polymerases are found mainly in vertebrates, and a few are found in plants and fungi. These polymerases have highly conserved regions that include **two helix-hairpin-helix** motifs that are imperative in the DNA-polymerase interactions. One motif is **located** in the 8 kDa domain that interacts with downstream DNA and

one motif is located in the thumb domain that interacts with the primer strand. Pol β, encoded by POLB gene, is required for short-patch base excision repair, a DNA repair pathway that is essential for repairing alkylated or oxidized bases as well as abasic sites. Pol λ and Pol μ, encoded by the POLL and POLM genes respectively, are involved in non-homologous end-joining, a mechanism for **rejoining** DNA double-strand **breaks due to hydrogen peroxide and** ionizing radiation, respectively. TdT is expressed only in lymphoid tissue, and adds "n nucleotides" to double-strand breaks formed during V(D)J recombination to promote immunological diversity. Polymerases α, δ and ε (alpha, delta, and epsilon) Pol α (alpha), Pol δ (delta), and Pol ε (epsilon) are members of Family B Polymerases and are the main polymerases involved with nuclear DNA replication. Pol α complex (pol α-DNA primase complex) consists of four subunits: the catalytic subunit POLA1, the regulatory subunit POLA2, and the small and the large primase subunits PRIM1 and PRIM2 respectively. Once primase has created the RNA primer, Pol α starts replication elongating the primer with ~20 nucleotides. Due to its high processivity, Pol δ takes over the leading and lagging strand synthesis from Pol α. Pol δ is expressed by genes POLD1, creating the catalytic subunit, POLD2, POLD3, and POLD4 creating the other subunits that interact with Proliferating Cell Nuclear Antigen (PCNA), which is a DNA clamp that allows Pol δ to possess processivity. Pol

ε is encoded by the POLE1, the catalytic subunit, POLE2, and POLE3 gene. It has been reported that the function of Pol ε is to extend the leading strand during replication, while Pol δ primarily replicates the lagging strand; however, recent **evidence??** suggested that Pol δ might have a role in replicating the leading strand of DNA as well. Pol ε's C-terminus "polymerase relic" region, despite being **un**necessary for polymerase activity, is thought to be essential to cell vitality. The C-terminus region is thought to provide a checkpoint before entering anaphase, provide stability to the holoenzyme, and add proteins to the holoenzyme necessary for initiation of replication. Pol ε has a larger "palm" domain that provides high processivity independently of PCNA. Compared to other Family B polymerases, the DEDD exonuclease family responsible for proofreading is inactivated in Pol α. Pol ε is unique in that it has two zinc finger domains and an inactive copy of another family B polymerase in its C-terminal. The presence of this zinc finger has implications in the origins of Eukaryota, which in this case is placed into the Asgard group with archaeal B3 polymerase. Polymerases η, ι and κ (eta, iota, and kappa) Pol η (eta), Pol ι (iota), and Pol κ (kappa), are Family Y DNA polymerases involved in the DNA repair by translesion synthesis and encoded by genes POLH, POLI, and POLK respectively. Members of Family Y have five common motifs to aid in binding the substrate and primer terminus and they all include the typical right hand

thumb, palm and finger domains with added domains like little finger (LF), polymerase-associated domain (PAD), or wrist. The active site, however, differs between family members due to the different lesions being repaired. Polymerases in Family Y are low-fidelity polymerases, but have been proven to do more good than harm as mutations that affect the polymerase can cause various diseases, such as skin cancer and Xeroderma Pigmentosum Variant (XPS). The importance of these polymerases is **evidenced** by the fact that gene encoding DNA polymerase η is referred as XPV, because loss of this gene results in the disease Xeroderma Pigmentosum Variant. Pol η is particularly important for allowing accurate translesion synthesis of DNA damage resulting from ultraviolet radiation. The functionality of Pol κ is not completely understood, but researchers have found two probable functions. Pol κ is thought to act as an extender or an inserter of a specific base at certain DNA lesions. All three translesion synthesis polymerases, along with Rev1, are recruited to damaged lesions via stalled replicative DNA polymerases. There are two pathways of damage repair **leading** researchers**???** to conclude that the chosen pathway depends on which strand contains the damage, the leading or lagging strand. Polymerases Rev1 and ζ (zeta) Pol ζ another B family polymerase, is made of two subunits Rev3, the catalytic subunit, and Rev7 (MAD2L2), which increases the catalytic function of the polymerase, and is involved in translesion synthesis. Pol ζ

lacks 3' to 5' exonuclease activity, is unique in that it can extend primers with terminal mismatches. Rev1 has three regions of interest in the BRCT domain, ubiquitin-binding domain, and C-terminal domain and has dCMP transferase ability, which adds deoxycytidine opposite lesions that would stall replicative polymerases Pol δ and Pol ε. These stalled polymerases activate ubiquitin complexes that in turn disassociate replication polymerases and recruit Pol ζ and Rev1. Together Pol ζ and Rev1 add deoxycytidine and Pol ζ extends past the lesion. Through a yet undetermined process, Pol ζ disassociates and replication polymerases reassociate and continue replication. Pol ζ and Rev1 are not required for replication, but loss of REV3 gene in budding yeast can cause increased sensitivity to DNA-damaging agents due to collapse of replication forks where replication polymerases have stalled. **Telomerase** is a ribonucleoprotein which functions to replicate ends of linear chromosomes since normal DNA polymerase cannot replicate the ends, or telomeres. The single-strand 3' overhang of the double-strand chromosome with the sequence 5'-TTAGGG-3' **recruits** telomerase. Telomerase acts like other DNA polymerases by extending the 3' end, but, unlike other DNA polymerases, telomerase does not require a template. The TERT subunit, an example of a reverse transcriptase, uses the RNA subunit to form the primer–template junction that allows telomerase to extend the 3' end of chromosome ends. The gradual decrease in

size of telomeres as the result of many replications over a lifetime are thought to be associated with the effects of aging.[13]:248–249 Polymerases γ, θ and ν (gamma, theta and nu) Further information: DNA polymerase nu Pol γ (gamma), Pol θ (theta), and Pol ν (nu) are Family A polymerases. Pol γ, encoded by the POLG gene, is the only mtDNA polymerase and therefore replicates, repairs, and has proofreading 3'–5' exonuclease and 5' dRP lyase activities. Any mutation that leads to limited or non-functioning Pol γ has a significant effect on mtDNA and is the most common cause of autosomal inherited mitochondrial disorders. Pol γ contains a C-terminus polymerase domain and an N-terminus 3'–5' exonuclease domain that are connected via the linker region, which binds the accessory subunit. The accessory subunit binds DNA and is required for processivity of Pol γ. Point mutation A467T in the linker region is responsible for more than one-third of all Pol γ-associated mitochondrial disorders. While many homologs of Pol θ, encoded by the POLQ gene, are found in eukaryotes, its function is not clearly understood. The sequence of amino acids in the C-terminus is what classifies Pol θ as Family A polymerase, although the error rate for Pol θ is more closely related to Family Y polymerases. Pol θ extends mismatched primer termini and can bypass abasic sites by adding a nucleotide. It also has Deoxyribophosphodiesterase (dRPase) activity in the polymerase domain and can show ATPase activity in close proximity to ssDNA. Pol ν (nu)

is considered to be the least effective of the polymerase enzymes. However, DNA polymerase nu plays an active role in homology repair during cellular responses to crosslinks, fulfilling its role in a complex with helicase. **Plants** use two Family A polymerases to copy both the mitochrondrial and plastid genomes. They are more similar to bacterial Pol I than they are to mamallian Pol γ. [54] Reverse transcriptase Retroviruses encode an unusual DNA polymerase called reverse transcriptase, which is an RNA-dependent DNA polymerase (RdDp) that synthesizes DNA from a template of RNA. The reverse transcriptase family contain both DNA polymerase functionality and RNase H functionality, which degrades RNA base-paired to DNA. An example of a retrovirus is HIV.

http://archive.wphna.org/wp-content/uploads/2015/04/2014-11-Hypothesis-Anne-Emanuelle-Birn-Rockefeller-and-Gates.pdf the relationship between the Rockefeller Foundation and the World Health Organization, Part I: 1940s-1960s. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(13)61013-2/fulltext https://beforeitsnews.com/conspiracy-theories/2020/03/coronavirus-traced-to-rothschilds-british-crown-2516412.html
https://libertygalaxy.com/rockefeller-globalism-using-health/

Sylvy ..Her first job after completing her master's degree was at the **National Cancer Institute.** There she became reacquainted with **Arthur Kornberg**, whom she had first met in Rochester, and who was by that time also at the **National Institutes of Health.** They **married** in 1943. // 1953 James **Watson** and Francis **Crick** published their classic paper positing the double-helix structure of DNA.

But **how** was the complex structure replicated? The Kornbergs, **along** with then postdoctoral fellows Robert Lehman **and** Maurice Bessman, and doctoral candidate Steven Zimmerman, delved into that question. As Lehman, a professor emeritus at Stanford University, noted, the group was frustrated by failed attempts to generate a DNA replication. **Sylvy** Kornberg discovered an enzyme that **degraded** an essential triphosphate, clearing the path for successful replication.

https://profiles.nlm.nih.gov/spotlight/wh/feature/biographical-overview

Denaturation is a process in which proteins or nucleic acids **lose** the quaternary structure, tertiary structure, and secondary structure which is present in their native state, by application of some **external** stress or compound such as a **strong acid** or base, a concentrated inorganic salt, an organic solvent (e.g., **alcohol** or **chloroform**), **radiation or heat**. If proteins in a living cell are denatured, this **results** in disruption of cell activity and possibly cell **death**. Protein denaturation is also a consequence of cell **death**. Denatured proteins can exhibit a wide range of characteristics, from conformational change and loss of solubility to aggregation due to the exposure of hydrophobic groups. Denatured proteins lose their 3D structure and therefore **cannot** function. Protein **folding** is

key to whether a globular or membrane protein can do its job correctly; it must be folded into the right shape to function. However, **hydrogen** bonds, which play a big part in folding, are rather **weak** and thus easily affected by heat, acidity, varying salt concentrations, and other stressors which can denature the protein. This is one reason why **homeostasis** is physiologically necessary in many life forms. This concept is **un**related to denatured alcohol, which is alcohol that has been mixed with additives to make it **unsuitable** for human consumption. When **food** is cooked, some of its proteins become denatured. This is why boiled eggs become hard and cooked meat becomes firm. A classic example of denaturing in proteins comes from egg **whites**, which are typically largely egg albumins in water. Fresh from the eggs, egg whites are transparent and liquid. Cooking the thermally unstable whites turns them opaque, forming an interconnected solid mass. The **same** transformation can be effected with a denaturing **chemical**. Pouring egg whites into a beaker of **acetone** will also turn egg whites translucent and solid. The skin that forms on curdled **milk** is another common example of denatured protein. The cold appetizer known as ceviche is prepared by **chemically "cooking"** raw fish and shellfish in an acidic citrus marinade, **without** heat. Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to protein aggregation. Proteins or Polypeptides are polymers of amino acids. A protein is **created** by

ribosomes that "read" RNA that is encoded by codons in the gene and assemble the requisite amino acid combination from the genetic instruction, in a process known as **translation**. The newly created protein strand then undergoes **post**translational modification, in which additional **atoms** or molecules are added, for example copper, zinc, or iron. Once this post-translational modification process has been completed, the protein begins to fold (sometimes spontaneously and sometimes with **enzymatic** assistance), curling up on itself so that hydro**phobic** elements of the protein are buried deep inside the structure and hydro**philic** elements end up on the outside. The final shape of a protein determines how it interacts with its environment. Protein folding consists of a balance between a substantial amount of **weak** intra-molecular interactions within a protein (Hydrophobic, electrostatic, and Van Der Waals Interactions) **and** protein-solvent interactions. As a result, this process is **heavily** reliant on environmental state that the protein resides in. These environmental conditions **include**, and are not limited to, temperature, salinity, pressure, and the solvents that happen to be involved. Consequently, any exposure to **extreme** stresses (e.g. heat or radiation, high inorganic salt concentrations, strong acids and bases) can **disrupt** a protein's interaction and inevitably lead to denaturation. When a protein is denatured, secondary and tertiary structures are **altered but** the peptide bonds**??** of the primary structure between the amino acids are **left**

intact. Since all structural levels of the protein determine its function, the protein can **no longer** perform its function **once** it has been denatured. This is in **contrast** to intrinsically **un**structured proteins, which are unfolded in their native state, **but still** functionally active and tend to fold upon binding to their biological target. In quaternary structure denaturation, protein sub-units are dissociated and/or the spatial arrangement of protein subunits is disrupted. Tertiary structure denaturation involves the disruption of: Covalent interactions between amino acid side-chains (such as disulfide bridges between cysteine groups) Non-covalent dipole-dipole interactions between polar amino acid side-chains (and the surrounding solvent) Van der Waals (induced dipole) interactions between nonpolar amino acid side-chains. In secondary structure denaturation, proteins **lose** all regular repeating patterns such as alpha-helices and beta-pleated sheets, and adopt a random coil configuration. Primary structure, such as the sequence of amino acids held together by covalent peptide bonds, is **not** disrupted by denaturation. Most biological substrates **lose** their biological function when denatured. For example, **enzymes** lose their activity, because the substrates can no longer bind to the active site, and because amino acid residues involved in stabilizing substrates' transition states are no longer positioned to be able to do so. The denaturing process and the associated loss of activity can be **measured** using

techniques such as dual-polarization interferometry, CD, QCM-D and MP-SPR.

By targeting proteins, **heavy** metals have been known to disrupt the function and activity carried out by proteins.

It is **important** to note that heavy metals fall into categories consisting of **transition** metals **as well as** a select amount of metalloid. These metals, when interacting with native, folded proteins, **tend** to play a role in obstructing their biological activity. This interference can be carried out in a different number of ways. These heavy metals can form a **complex** with the functional side chain groups present in a protein or form bonds to free thiols. **Heavy** metals also play a role in oxidizing amino acid side chains present in protein. Along with this, when interacting with **metallo**proteins, heavy metals can dislocate and replace key metal ions??. As a result, heavy metals can interfere with folded proteins, which can strongly deter protein stability and activity. In many cases, denaturation is **reversible??** (the proteins can regain their native state when the denaturing influence is removed). This process can be called **re**naturation. This understanding has led to the notion that all the information needed for proteins to assume their native state was encoded in the primary structure of the protein, and hence in the DNA that codes for the protein, the so-called "Anfinsen's thermodynamic **hypothesis**". Denaturation can also be irreversible. This irreversibility is typically a kinetic, **not** thermodynamic irreversibility,

as generally when a protein is folded it has **lower free** energy. Through kinetic irreversibility, the fact that the protein is stuck in a local minimum can stop it from ever refolding after it has been irreversibly denatured. Protein denaturation due to pH Denaturation can also be caused by **changes** in the pH which can affect the chemistry of the amino acids and their residues. The **ionizable** groups in amino acids are able to **become** ionized when changes in pH occur. A pH change to **more acidic or** more basic conditions can induce unfolding. **Acid**-induced unfolding often occurs between pH 2 and 5, **base**-induced unfolding usually requires pH 10 or higher.
Nucleic acids (including RNA and DNA) are nucleotide **polymers** synthesized by **polymerase** enzymes during either transcription or DNA replication. Following 5'-3' synthesis of the backbone, individual nitrogenous bases are capable of interacting with one another via hydrogen bonding, thus allowing for the formation of higher-order structures. Nucleic acid denaturation occurs when hydrogen bonding between nucleotides is disrupted, and results in the separation of **previously** annealed strands. For example, **de**naturation of DNA due to **high** temperatures **results** in the disruption of **Watson** and **Crick** base pairs and the separation of the double stranded helix into two single strands. Nucleic acid strands are capable of **re**-annealling when "normal" conditions are restored, **but** if restoration occurs too quickly, the nucleic

acid strands may **re**-anneal **imperfectly** resulting in the improper pairing of bases.

Biologically-induced denaturation.
The non-covalent interactions between **anti**parallel strands in DNA can be broken , in order to "open" the double helix when biologically important mechanisms such as DNA replication, transcription, DNA repair or protein binding are set to occur. The **area** of partially separated DNA is known as the denaturation **bubble**, which can be **more** specifically defined as the opening of a DNA double helix through the coordinated separation of base pairs. The **first model** that **attempted** to describe the thermodynamics of the denaturation bubble was introduced in 1966 **and** called the Poland-Scheraga Model. This model describes the denaturation of DNA strands as a **function** of temperature. As the temperature increases, the hydrogen bonds between the Watson and Crick base pairs are increasingly disturbed and "denatured loops" begin to form. However, the Poland-Scheraga Model is now considered elementary because it **fails** to account for the confounding implications of DNA sequence, chemical composition, stiffness and torsion. Recent thermodynamic studies have **inferred** that the **lifetime** of a singular denaturation bubble **ranges** from 1 **micro**second to 1 **milli**second. This information is **based** on established timescales of DNA replication and transcription. Biophysical and biochemical research

studies are being performed **to more fully** elucidate the thermodynamic details of the denaturation bubble.

Denaturation due to **chemical** agents**.**
With polymerase chain reaction (PCR) being among the most popular contexts in which DNA denaturation is desired, **heating** is the most frequent method of denaturation. Other than denaturation by heat, nucleic acids can undergo the **denaturation process through various chemical agents such as** formamide, guanidine, sodium salicylate, dimethyl sulfoxide (DMSO), propylene glycol, and urea. These chemical denaturing agents **lower** the melting temperature (Tm) **by** competing for hydrogen bond donors and acceptors with **pre**-existing nitrogenous base pairs. Some agents are even able to **induce** denaturation at room temperature. For example, **alkaline** agents (e.g. NaOH) have been shown to denature DNA by changing pH and removing hydrogen-bond contributing protons. These denaturants have been employed to make Denaturing Gradient Gel Electrophoresis gel (DGGE), which **promotes** denaturation of nucleic acids in order to eliminate the influence of nucleic acid **shape** on their electrophoretic mobility.

Chemical denaturation as an alternative.
The optical activity (absorption and scattering of light) **and** hydrodynamic properties (translational diffusion, sedimentation coefficients, and rotational correlation

times) of **formamide** denatured nucleic acids are similar to those of heat-denatured nucleic acids. Therefore, depending on the **desired** effect, chemically denaturing DNA can provide a **gentler** procedure for denaturing nucleic acids **than** denaturation induced by heat. Studies comparing different denaturation methods such as heating, beads mill of different bead sizes, probe sonification, and chemical denaturation show that chemical denaturation can provide **quicker** denaturation compared to the other physical denaturation methods described. Particularly in cases where **rapid** renaturation is desired, chemical denaturation agents can provide an ideal alternative to heating. For example, DNA strands denatured with **alkaline** agents such as NaOH **renature** as soon as **phosphate** buffer is **added**.

Denaturation due to **air**.

Small, electronegative molecules such as **nitrogen** and oxygen, which are the primary gases in air, significantly impact the ability of surrounding molecules to participate in hydrogen bonding. These molecules **compete?** with surrounding hydrogen bond acceptors for hydrogen bond donors, therefore acting as "hydrogen bond **breakers**" and **weakening** interactions between surrounding molecules in the environment. **Anti**parellel strands in DNA double helices are **non**-covalently bound by hydrogen bonding between Watson and Crick base pairs; nitrogen and oxygen therefore maintain the potential to

weaken the integrity of DNA when exposed to **air**. As a result, DNA strands exposed to air require **less** force to separate and exemplify lower melting temperatures.

Applications.

Many laboratory techniques **rely** on the ability of nucleic acid strands **to** separate. By understanding the properties of nucleic acid denaturation, the following methods were **created**: PCR , Southern blot , Northern blot , DNA Sequencing.

Denaturants.

Protein denaturants Acids

Acidic protein denaturants include: Acetic acid.. Trichloro**acetic** acid 12% in water . Sulfosalicylic acid.

Bases

Bases work **similarly** to acids in denaturation. They include: Sodium bicarbonate.

Solvents

Most organic solvents are denaturing, including:Ethanol, Alcohol.

Cross-linking reagents.

Cross-linking agents for proteins include: Formaldehyde, Glutaraldehyde.

Chaotropic agents.

Chaotropic agents include: Urea 6 – 8 mol/l , Guanidinium chloride 6 mol/l , Lithium perchlorate 4.5 mol/l , Sodium dodecyl sulfate.

Disulfide bond reducers
Agents that break disulfide bonds by reduction include: 2-Mercaptoethanol , Dithiothreitol TCEP (tris(2-carboxyethyl)phosphine).

Chemically reactive agents.
Agents such as Hydrogen Peroxide, Elemental Chlorine, Hypochlorous Acid(Chlorine Water),Bromine, Bromine Water, Iodine,Nitric & Oxidising Acids, and Ozone react with sensitive moieties such as sulfide/Thiol, activated aromatic rings (phenylalanine) in effect , **damage** the protein and render it **useless**.

Other Mechanical agitation
Picric acid , Radiation , Temperature.

Nucleic acid denaturants.
Chemical Acidic nucleic acid denaturants include: Acetic acid , HCl , Nitric Acid.
Basic nucleic acid denaturants include: NaOH . Other nucleic acid denaturants include: DMSO , Formamide , Guanidine , Sodium salicylate , Propylene glycol , Urea.

Physical denaturation.

Thermal denaturation, Beads mill , Probe sonication , Radiation.

A reverse transcriptase (RT) is an **enzyme** used to generate complementary DNA (cDNA) from an RNA template, a process termed reverse transcription. Reverse transcriptases are used by retroviruses**???** to replicate their genomes, **by** retrotransposon mobile genetic elements to proliferate within the host genome, by **eukaryotic** cells to **extend** the telomeres at the ends of their linear chromosomes, and by some **non**-retroviruses such as the hepatitis B virus, a member of the Hepadnaviridae, which are dsDNA-RT viruses. Retroviral RT has **three** sequential biochemical activities: RNA-dependent DNA polymerase activity, ribonuclease H (RNAse H), and DNA-dependent DNA polymerase activity. **Collectively**, these activities enable the enzyme to **convert** single-stranded RNA into double-stranded cDNA. In retroviruses **and** retrotransposons, this cDNA can then **integrate** into the host genome, **from which new** RNA copies can be made via host-cell transcription. The **same** sequence of reactions is **widely** used in the laboratory to convert RNA to DNA for use in molecular cloning, RNA sequencing, polymerase chain reaction (PCR), or genome analysis. **Reverse transcriptases were discovered by Howard Temin** at the University of Wisconsin–Madison in Rous **sarcoma** virions and

independently **isolated** by **David Baltimore** in **1970** at **MIT** from two RNA tumour viruses: murine leukemia virus **and** again Rous sarcoma virus. For their achievements, they shared the 1975 Nobel Prize in Physiology or Medicine (with Renato Dulbecco). **Well-studied reverse** transcriptases include: HIV-1 reverse transcriptase **from** human immunodeficiency virus type 1 (PDB: 1HMV) has **two** subunits, which have respective molecular weights of **66** and 51 kDa. M-MLV reverse transcriptase from the Moloney murine leukemia virus is a single 75 kDa monomer. AMV reverse transcriptase from the avian myeloblastosis virus also has two subunits, a 63 kDa subunit and a 95 kDa subunit. Telomerase reverse transcriptase that maintains the telomeres of eukaryotic chromosomes. The enzymes are encoded and used by **viruses** that use reverse transcription as a step in the process of replication. Reverse-transcribing RNA viruses, such as retroviruses, use the enzyme to reverse-transcribe their RNA genomes into DNA, which is then integrated into the host genome and replicated along with it. Reverse-transcribing DNA viruses, such as the hepadnaviruses, can allow RNA to serve as a template in assembling and making DNA strands. **HIV infects humans with the use of this enzyme. Without** reverse transcriptase, the viral genome would **not** be able to incorporate into the host cell, resulting in failure to replicate.

Process of reverse transcription or retrotranscription Reverse transcriptase creates double-stranded DNA from an RNA template. In virus species with reverse transcriptase lacking DNA-dependent DNA polymerase activity, creation of double-stranded DNA can **possibly** be done by host-encoded DNA polymerase δ, **mistaking** the viral DNA-RNA for a **primer** and synthesizing a double-stranded DNA by similar mechanism as in primer removal, where the newly synthesized DNA displaces the original RNA template. The process of reverse transcription, **also** called retrotranscription or **retrotras**, **is extremely error-prone, and it is during this step that mutations may occur.** Such mutations may cause drug resistance. Retroviruses, **also** referred to as class VI ssRNA-RT viruses, are RNA reverse-transcribing viruses with a DNA intermediate. Their genomes consist of **two** molecules of positive-sense single-stranded RNA with a 5' cap & 3' polyadenylated tail.**Examples** of retroviruses include the human immunodeficiency virus (HIV) and the human T-lymphotropic virus (HTLV).

Creation of double-stranded DNA occurs in the cytosol as a series of these steps: Lysyl tRNA acts as a primer and hybridizes to a complementary part of the virus RNA genome **called** the primer binding site or PBS. Reverse transcriptase then adds DNA nucleotides onto the 3' end of the primer, synthesizing DNA complementary to the U5 (non-coding region) and R region (a direct repeat

found at both ends of the RNA molecule) of the viral RNA. A domain on the reverse transcriptase enzyme called RNAse H degrades the U5 and R regions on the 5’ end of the RNA. The tRNA primer then "jumps" to the 3’ end of the viral genome, and the newly synthesised DNA strands hybridizes to the complementary R region on the RNA. The complementary DNA (cDNA) added in (2) is further extended. The majority of viral RNA is degraded by RNAse H, leaving only the PP sequence. Synthesis of the second DNA strand begins, using the remaining PP fragment of viral RNA as a primer. The tRNA primer leaves and a "jump" happens. The PBS from the second strand hybridizes with the complementary PBS on the first strand. Both strands are **extended** to form a complete double-stranded DNA copy of the original viral RNA genome, which can **then** be incorporated into the host's genome by the enzyme integrase. **Creation of double-stranded DNA also involves strand transfer,** in which there is a translocation of short DNA product from initial RNA-dependent DNA synthesis to acceptor template regions at the other end of the genome, which are later reached and processed by the reverse transcriptase for its DNA-dependent DNA activity. Retroviral RNA is arranged in 5’ terminus to 3’ terminus. The site where the primer is annealed to viral RNA is **called** the primer-binding site (PBS). The RNA 5’end to the PBS site is **called** U5, and the RNA 3’ end to the PBS is **called** the **leader**. The tRNA primer is unwound between 14 and 22

nucleotides and forms a base-paired duplex with the viral RNA at PBS. The fact that the PBS is located near the 5' terminus of viral RNA is **unusual** because reverse transcriptase synthesize DNA from 3' end of the primer in the 5' to 3' direction (with respect to the newly synthesized DNA strand). Therefore, the primer and reverse transcriptase must be **relocated** to 3' end of viral RNA. In order to accomplish this **re**position, **multiple steps and various enzymes including DNA polymerase, ribonuclease H(RNase H) and polynucleotide unwinding are needed.** The HIV reverse transcriptase also has ribonuclease activity that **degrades** the viral RNA during the **synthesis** of cDNA, as well as DNA-dependent DNA polymerase activity that copies the sense cDNA strand into an **anti**sense DNA to form a double-stranded viral DNA intermediate (vDNA). In cellular life Self-replicating stretches of eukaryotic genomes known as retrotransposons utilize reverse transcriptase to move from one position in the genome to another via an RNA intermediate. They are found **abundantly** in the genomes of plants **and** animals. **Telomerase** is another reverse transcriptase found in many eukaryotes, including humans, which carries its own RNA template; this RNA is used as a template for DNA replication. Initial reports of reverse transcriptase in prokaryotes came as far back as **1971** (Beljanski et al., 1971a, 1972). These have since been broadly described as **part of bacterial** Retrons, distinct sequences that code for reverse transcriptase, and

are used in the synthesis of msDNA. In order to **initiate** synthesis of DNA, a **primer** is needed. In **bacteria**, the primer is synthesized during replication. Valerian Dolja of Oregon State argues that **viruses**, due to their diversity, have played an evolutionary role in the development of cellular life, with reverse transcriptase playing a **central** role.

The reverse transcriptase employs a "right hand" structure **similar** to that found in other viral nucleic acid polymerases. In **addition** to the transcription function, retroviral reverse transcriptases have a **domain** belonging to the RNase H family, which is **vital** to their replication. By degrading the RNA template, it allows the other strand of DNA to be synthesized. Some fragments from the digestion **also** serves as the primer for the DNA polymerase (either the **same** enzyme **or** a host protein), responsible for making the other (plus) strand.

There are **three** different replication systems during the life cycle of a retrovirus. **First** of all, the reverse transcriptase **synthesizes** viral DNA **from** viral RNA, and then **from** newly made complementary DNA strand. The **second** replication process occurs when host cellular DNA polymerase replicates the integrated viral DNA. **Lastly**, RNA polymerase II transcribes the proviral DNA into RNA, which will be packed into virions. Therefore, **mutation** can occur during one or all of these replication steps. Reverse transcriptase has a **high error rate** when transcribing RNA into DNA since, **unlike** most other

DNA polymerases, it has **no proofreading ability.** This **high error rate allows mutations to accumulate at an accelerated rate relative to** proofread forms of replication. The commercially available reverse transcriptases produced by **Promega** are quoted by their manuals as having error rates in the range of 1 in 17,000 bases for AMV and 1 in 30,000 bases for M-MLV. Other than creating single-nucleotide polymorphisms, reverse transcriptases have **also** been shown to be involved in processes **such as** transcript fusions, exon shuffling and creating artificial antisense transcripts. It has been **speculated** that this template switching activity of reverse transcriptase, which can be demonstrated completely in **vivo**, **may** have been **one of the causes** for finding **several thousand unannotated transcripts in the genomes of model organisms.**

Antiviral drugs

Further information: Reverse-transcriptase **inhibitor.**

As HIV uses reverse transcriptase to copy its genetic material and **generate new** viruses (part of a retrovirus proliferation circle), **specific** drugs have been designed to disrupt the process and thereby suppress its growth. **Collectively**, these drugs are known as reverse-transcriptase **inhibitors** and include the nucleoside and nucleotide analogues **zidovudine** (trade name **Retrovir**), lamivudine (Epivir) **and** tenofovir (Viread), as well as

non-nucleoside **inhibitors**, such as nevirapine (Viramune).

Molecular biology
Reverse transcription polymerase chain reaction.
Reverse transcriptase is commonly used in **research** to apply the polymerase chain reaction technique to RNA in a technique called reverse transcription polymerase chain reaction (RT-PCR). The **classical** PCR technique can be applied **only** to DNA strands, **but**, with the help of reverse transcriptase, RNA can be transcribed into DNA, thus making PCR analysis of RNA molecules possible. Reverse transcriptase is used **also** to create cDNA libraries from mRNA. The commercial availability of reverse transcriptase greatly improved knowledge in the area of molecular biology, as, along with **other** enzymes, it allowed scientists to clone, sequence, and characterise RNA.

Reverse transcriptase has **also** been employed in **insulin** production. By inserting eukaryotic mRNA for **insulin** production **along** with reverse transcriptase **into bacteria**, the mRNA could be inserted into the prokaryote's genome. **Large amounts of insulin can then be created, sidestepping** the need to harvest **pig** pancreas **and** other such traditional sources. Directly inserting eukaryotic DNA into bacteria would **not** work because it carries introns, so would not translate successfully using the

bacterial ribosomes. Processing in the eukaryotic cell during mRNA production **removes** these introns to provide a suitable template. Reverse transcriptase converted this **edited** RNA back into DNA so it could be incorporated in the genome.

POLYMERASE CHAIN REACTION .
SUSAN J. KARCHER, in 'Molecular Biology', 1995
Polymerase chain reaction (PCR) is the in **vitro** amplification of specific sequences of nucleic acid. The processes of PCR and the enzyme DNA polymerase were named by Science magazine **as** the 1989 "Molecule of the Year" because they were likely to have the greatest influence on history (Guyer and Koshland, 1989). Science said that PCR was "revolutionizing the approaches researchers are taking to , many problems in biology."

The basic steps of PCR are (1) the denaturation by heating of a template DNA molecule to be copied, (2) the annealing of pairs of oligonucleotides of specific sequences (primers, typically 10–14 nt long) chosen to be homologous to sequences within the template DNA molecule, and (3) the extension by DNA polymerase from the primers to copy the template DNA molecule. dNTPs must be present in the reaction. These **three** steps are **repeated** many times (for many cycles) to amplify the template DNA. If in each cycle one copy is made of each

of the strands of the template, the number of DNA molecules produced doubles each cycle. Because of this doubling, at the end of 20 cycles, more than one million copies of the template DNA are made. The **first** experiments in PCR used **Escherichia** coli DNA polymerase I **inactivated** by the heat treatment to denature DNA template molecules. The polymerase had to be added repeatedly to the reaction. What made this in vitro DNA amplification so efficient was the discovery of heat-stable DNA polymerases such as Taq DNA polymerase from Thermus aquaticus, a eubacterium that grows in the elevated temperatures of aquatic **hot** springs. With heat-stable DNA polymerases, all the components can be added at the start of the reaction. The reaction is then cycled through the different temperatures that allow amplification to occur. The heat-stable DNA polymerases gradually **lose** activity over the course of the cycles. Although the temperature **and** time for each of the steps of 1 cycle described above will be **varied** according to the sequence of the primers used, a general example of the steps is (1) Double-stranded DNA to be amplified is denatured by high temperature (i.e., 95°C for 2 min). (2) The temperature is reduced to 55°C for 2 min to allow specific **primers** to hybridize to the target sequences. (3) The temperature is increased to 75°C for 2 min. The heat-stable DNA polymerase begins DNA synthesis at the primer and synthesizes the other DNA strand. This results in the duplication of the template DNA. Many cycles of

steps 1–3 are repeated to amplify the template DNA many times. 1. Denature template, 99°C, 2 min. 2. Anneal primers to template, 55°C, 2 min. 3. Extension of the primers by heat stable DNA polymerase, 72°C, 2 min. Steps 1 through 3 are then repeated many times. Using Taq DNA polymerase, the PCR amplification generally works best **if** primers hybridize to regions of the template not more than 2–4 kb apart. However, it is possible for Taq DNA polymerase to copy fragments up to 10 kb long. Taq DNA polymerase **lacks** a proofreading activity and can make a number of **errors** while copying the template. Because Taq DNA polymerase synthesis is **error prone,** care should be taken if the PCR amplification product is to be cloned. To clone a PCR product, several fragments from different PCR amplification reactions should be cloned and the sequences compared **to be certain that sequence errors have not been cloned.**

Other heat-stable DNA polymerases have been identified and characterized. For example, Vent DNA polymerase is isolated from the **archaebacterium** Thermococcus litoralis, which lives near thermal vents in the ocean floor and grows at temperatures of up to 98°C. Vent DNA polymerase (New England BioLabs) can produce PCR products up to 13 kb long and has a **higher** fidelity of DNA replication than Taq DNA polymerase. Another thermal-stable polymerase that has been characterized is Deep Vent DNA polymerase, isolated from Pyrococcus

species GB-D that grow at **ocean depths** of 2,010 m where vent temperatures can be **104**°C**???**. Deep Vent DNA polymerase also has **very high** sequence fidelity of synthesis. The polymerase chain reaction is very **sensitive**. It is possible to amplify a **single** or a very small number of molecules. This fact is **simultaneously** the great advantage of PCR **and a problem** with the technique. Because PCR is **extremely sensitive,** great care must be taken to avoid contamination of samples to be amplified by other DNA. Micropipettors with tips that contain a **filter** can be used so that the material being pipeted will **not ???** be contaminated from a previously contaminated pipet. Some of the myriad of applications of the PCR technique include the following: 1. Disease **diagnosis**. a. Because a specific sequence can be amplified **greatly**, much less clinical material is needed to make a diagnosis. b. PCR can be used to detect pathogens that are difficult to **culture**, such as the **causative** agents for Lyme disease or for AIDS. c. PCR can be used for **cancer** diagnosis. 2. **Forensics**. DNA sequences from trace amounts of biological material such as semen, blood, and **hair** roots can be amplified. Probes for regions that show hypervariability in the population, and therefore make good **markers** to identify the **source** of the DNA, are available. PCR can be used to evaluate evidence at the scene of a crime, to help to identify missing persons, and in paternity cases. 3. Matching donor and recipient tissues for organ transplants. 4. Basic research. a. A comparison

of sequence homology of conserved genes in different organisms can be made. It is **even** possible to study extinct organisms using samples of material from bones or museum specimens. The DNA used for PCR amplification can be **partially** degraded. As long as a few DNA molecules are **intact** between the two primers, amplification can occur. b. In developmental biology, PCR is very sensitive and can be used to examine which genes are turned **on** in early development (which mRNAs are made). Even if only a few transcripts are made, PCR can detect them**???**. c. Because PCR is so rapid and easy to do, it may **replace** cloning as the amplification method of choice to obtain **large** amounts of material for sequencing. One **potential drawback** to PCR is that one must have some sequence information about the piece of DNA to be amplified to make the appropriate specific primers. In the standard PCR, **one needs sequence information from both ends of** the DNA to be amplified. **Inverse** PCR is a variation of the standard PCR that requires sequence information **from only one part of** the DNA to be amplified. In **inverse** PCR, the DNA flanking (outside) the one primer region is amplified. DNA to be amplified is cut with a restriction endonuclease and circularized at the restriction endonuclease site. Primers to direct DNA synthesis outward **from one known** sequence are then amplified by PCR.

Inverse polymerase chain reaction. This process results in the amplification of the DNA outside (flanking) a known

sequence. DNA is digested with a restriction endonuclease and circularized. A pair of primers directed outward from **each end** of the known sequence is used for PCR. Many **variations** on the basic PCR procedure have been devised for specific uses.

As stated in Science (Appenzeller, 1990), PCR created a revolution and "new uses for PCR are developing almost as rapidly as the Taq polymerase can replicate target sequences."

Mullis (1990) has written an interesting personal account of his **initial** PCR work. The tremendous significance of this discovery was recognized by the awarding of the 1993 Nobel Prize in Chemistry to **Kary B. Mullis** for invention of the PCR method (Dagani, 1993). The 1993 Nobel Prize in Chemistry was awarded jointly to Michael Smith for his work on oligonucleotide-based site-directed mutagenesis.

Polymerase Chain Reaction (PCR) Robert K. Delong, Qiongqiong Zhou, in Introductory Experiments on Biomolecules and their Interactions, 2015

Introduction

Polymerase chain reaction (PCR), invented by scientist Kary Mullis in the early 1980s, and for which he won a Nobel Prize in 1993, allows researchers to amplify pieces of DNA by several orders of magnitude. This technique has revolutionized many aspects of current research,

including DNA cloning and sequencing, functional analysis of genes, the diagnosis of hereditary or infectious diseases, the identification of genetic fingerprints, and so on. The basic components of a PCR reaction include a DNA template, primers, nucleotides, DNA polymerase, and a buffer. The DNA template usually is your sample DNA, which contains the DNA region to be amplified. It could be plasmid DNA, genomic DNA, or even a small amount of tissue. The template DNA is typically given at very low concentrations in a PCR reaction, 1 pg–1 ng of plasmid or viral templates, 1 ng–1 μg of genomic templates. Primers are short oligonucleotides of DNA (typically 15–25 nucleotides) with a specific sequence that is custom synthesized on an automated DNA synthesizer. Today primers are typically obtained by providing the required sequence to one of many companies that specialize in oligomer provision. Primer **design is critical** for a successful PCR reaction. In general, the two primers match to the two ends of the segment of DNA you want to amplify. Through complementary base pairing, the 5 end primer matches to the top strand at one end of your segment of interest, and the other primer matches to the bottom strand at the other end. Besides the complementary sequence on the primer, you can **also add an extra sequence** (such as restriction cutting site, tag sequences, and so on), on the 5 end of the primer, depending on the needs of the experiment.

Primer design

DNA polymerase **is an enzyme complex** that amplifies DNA during cell cycle in a living organism. The DNA polymerase used in a PCR reaction usually can tolerate high temperature (95°C), the temperature necessary to separate two complementary strands of DNA in a test tube. For example, the Taq polymerase purified from Thermus aquaticus, a strain of bacteria living in a hot spring, can survive near boiling temperatures, and it works quite well at 72°C. Nucleotides are the building blocks for making the DNA molecules. In PCR reactions, a **mixture of four types of nucleotides** (ATP, CTP, GTP, TTP; known as dNTPs) **will be added.** DNA polymerase grabs the complementary nucleotides that are floating in the liquid around it and attaches them to the 3 end of a primer and pairing with the template DNA. PCR buffers help to maintain the right pH during the reaction cycles and provide necessary ions for enzymes to work. A typical PCR buffer stock solution is provided in a 10X or 5X format; you would need to **dilute** it to 1X in the PCR reaction.

Components and Their Concentrations in a Typical PCR Reaction

Components Stock conc. Working conc. Vol. to add DNA template 10 μg/mL 20 ng/reaction 2.0 μL **Primer** 1 20 μM 1 μM 2.5 μL **Primer** 2 20 μM 1 μM 2.5 μL **dNTP** 10 mM 0.2 mM 1.0 μL **Taq** polymerase 1 U/μL 1 U/reaction

1.0 µL PCR **buffer** 10X 1X 5.0 µL **water** 36.0 µL reaction vol. 50.0 µL PCR cycles: **A typical PCR procedure takes place in an automated thermal cycler machine consisting of a series of 20–40 repeated cycles with consistent temperature changes.** In each cycle, there are **three** steps called the denaturation step (94°C–98°C), annealing step (50°C–65°C), and elongation step (72°C). On **top** of the cycling steps, there are usually a single temperature step called the **initialization** step at a high temperature (> 90°C) **before** the cycling starts, a **final** elongation step (70°C–74°C), and a **final** hold step (4°C) **after** the cycling ends.
General Guidelines for Programing a Typical PCR Reaction in an Automated Thermal Cycler Machine. Cycles Time Temperature Steps 1 5 min 95°C Initialization ~30 30 sec 94°C Denaturation 30 sec 55°C–65°C Annealing ~ 1 min/kb 72°C Elongation 1 10 min 72°C Final elongation 1 ∞ 4°C Final hold Introduction Ayaz Najafov, Gerta Hoxhaj, in PCR Guru, 2017

A bit of history
Polymerase chain reaction (PCR) was invented by Dr. Kary Mullis in 1983. At that time, he was working at **Cetus** Corporation, one of the first biotechnology companies. For his invention, Dr. Mullis received a $10,000 bonus from Cetus. In 1992, Dr. Mullis sold the patent for PCR and Taq polymerase to Hoffmann La **Roche** for $300 million. In 1993, he received a Nobel

Prize in Chemistry “for his invention of the polymerase chain reaction method”.
Polymerase Chain Reaction Polymerase chain reaction (PCR) assays have been developed over the last 2 decades for detection of T. pallidum subsp. pallidum nucleic acids. Although designed to diagnose **syphilis** infection, the analysis of the available T. pallidum and **non**-T. pallidum strain genomes confirmed that these methods could also be applied to detect nucleic acids from all of these treponemal subspecies, but without differentiating them. On the other hand, PCR assays to distinguish nonvenereal T. pallidum subspecies have also been evaluated. T. p. pertenue and T. p. endemicum genetic signatures have been identified. Epstein-Barr Virus (Infectious Mononucleosis, Epstein-Barr Virus–Associated Malignant Diseases, and Other Diseases) Eric C. Johannsen, Kenneth M. Kaye, in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), 2015 . Central Nervous System Lymphoma in Acquired Immunodeficiency Syndrome Polymerase chain reaction detection of EBV DNA in CSF has been useful in the diagnosis of CNS lymphoma in patients with HIV.388-391 **Nearly all primary CNS lymphomas in HIV disease are EBV associated,** as discussed previously. Whereas patients with HIV **without** CNS lymphoma **rarely** have detectable EBV DNA in CSF, EBV DNA is frequently detected when CNS lymphoma is present. Therefore, CSF PCR for EBV used

in conjunction with radiologic studies may reduce the need for **brain** biopsy in certain instances. Quantification of EBV DNA in CSF may also be useful for monitoring the effects of CNS lymphoma therapy.392 Clinical Neuroscience Dennis R. Mosier MD, PhD, in Neurology Secrets (Fifth Edition), 2010

What is the polymerase chain reaction? Polymerase chain reaction (PCR) is a process that is used to amplify a region of DNA, thus allowing it to be detected with high sensitivity. It **requires knowledge** of the DNA sequence on **either** side of a target region (flanking sequence). DNA **primers** matching the flanking sequence are used to **initiate** copying of the target region DNA , by a heat-stable DNA polymerase. The resulting DNA strands are then heated to separate them and allow the primers to copy again, synthesizing new strands. This cycle is repeated until the desired **amplification (repeated copying) of the target region DNA** is achieved.

Bordetella pertussis and Pertussis (Whooping Cough) Sylvia H. Yeh, ChrisAnna M. Mink, in Netter's Infectious Diseases, 2012

Polymerase chain reaction (PCR) is an amplified molecular testing tool that often detects sequences in the pertussis toxin gene. In multiple trials evaluating NP samples, PCR has demonstrated **higher sensitivity than**

culture, as well as a more rapid turnaround time. The **deficiencies** of PCR include **lack of standardization, limited availability, and the potential for contamination yielding false-positive results.** Given the greater sensitivity and rapidity of PCR as compared with culture, PCR is gaining **favor** for diagnosing pertussis. Although many **commercial** laboratories offer PCR testing for B. pertussis, **no Food and Drug Administration (FDA)–approved assays are currently available.**???

Community-Acquired Bacterial Pneumonia Paulo J.C. Marostica MD, Renato T. Stein MD, MPH, PhD, in Kendig & Chernick's Disorders of the Respiratory Tract in Children (Eighth Edition), 2012
Polymerase chain reaction (PCR) has been used **more recently as a diagnostic tool in respiratory** infections. It may be applied to **specimens from respiratory secretions, lung aspirate samples, or blood.** Respiratory viruses, M. pneumoniae, C. pneumoniae, and other bacteria can be**??** diagnosed by PCR. It is a good diagnostic tool in research and can be used by clinicians in special situations, **but it does not differentiate carrier state from disease.** It is possible that quantitative PCR may solve these problems **if cutoff levels can be adequately defined.** More details of these and other tests for viral detection can be found in **other** chapters.

Polymerase Chain Reaction for Knocking Out Genes Sarah Maddocks, Rowena Jenkins, in Understanding PCR, 2017

Polymerase chain reaction (PCR) can be utilized as a molecular tool for functional analysis of genes and can be used in concert with protein and gene expression to thoroughly**???** describe the role of a given gene or genes. This chapter will describe how PCR can be used to prepare **construct** that are necessary to generate knockout mutations and how to assign function by complementing gene mutations using **Kochs'** molecular postulates. This chapter uses prokaryotes as a **model** for generating knockout mutations, and the procedures described will need to be adapted for **single** celled eukaryotes such as yeast and for more complex organisms.

Integrating Genomics into Pharmacy Education and Practice Daniel A. Brazeau, Gayle A. Brazeau, in Pharmacogenomics, 2013

Quantitative Polymerase Chain Reaction (Q-PCR) – an advancement in standard polymerase chain reaction (PCR) that allows for both the detection and quantification of very rare DNA targets by **assessing** the amplification process with **each** PCR cycle (in real time), this quantification is target specific from samples derived from the mRNA pool of a few hundred cells (assessing the transcriptome) or from genomic DNA from a buccal

swab (genetic testing). Q-PCR is the method of choice for assessing the expression levels of any gene or genes in the human genome. Because the technology is based upon the polymerase chain reaction quantification of gene expression, it can be accomplished from minute quantities of tissue, thus allowing for the localization of gene expression. Q-PCR is **routinely used to validate the findings of larger microarray or sequencer-based** genome expression studies.
https://www.sciencedirect.com/topics/neuroscience/polymerase-chain-reaction

Polymerase chain reaction, or PCR, is a technique to make many copies of a specific DNA region **in vitro (in a test tube rather than an organism).** PCR relies on a thermostable DNA polymerase, Taq polymerase, and **requires** DNA **primers** designed **specifically** for the DNA region of interest. In PCR, the reaction is repeatedly cycled through a series of temperature changes, which allow many copies of the target region to be produced. PCR has many research and practical applications. It is routinely used in DNA cloning, medical diagnostics, and forensic analysis of DNA.
What is PCR? Polymerase chain reaction (PCR) is a common laboratory technique used to make many copies (millions or **billions**!) of a particular region of DNA. This DNA region **can be anything the experimenter is**

interested in. For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects. Typically, the goal of PCR is to make enough of the target DNA region that it can be analyzed or used in some other way. For instance, DNA amplified by PCR may **be sent for** sequencing, visualized by gel electrophoresis, or cloned into a plasmid for further experiments. PCR is used in many areas of biology and medicine, including **molecular** biology research, medical diagnostics, and even some branches of **ecology**.
Taq polymerase ..Like DNA replication in an organism, PCR requires a DNA polymerase enzyme that makes new strands of DNA, using existing strands as templates. The DNA polymerase typically used in PCR is called Taq polymerase, after the heat-tolerant bacterium from which it was isolated (Thermus aquaticus). T. aquaticus lives in hot springs and hydrothermal vents. Its DNA polymerase is very heat-stable and is **most active** around 70 °\text C70°C70, °, start text, C, end text (a temperature at which a human or E. coli DNA polymerase would be nonfunctional). This heat-stability makes Taq polymerase **ideal** for PCR. As we'll see, **high** temperature is used **repeatedly** in PCR to denature the template DNA, or separate its strands. PCR **primers** Like other DNA polymerases, Taq polymerase can **only** make DNA **if it's given a primer,** a short sequence of nucleotides that provides a **starting** point for DNA synthesis. In a PCR

reaction, the **experimenter determines** the region of DNA that will be copied, or amplified, **by the primers** she or he chooses. PCR **primers are short pieces of single-stranded DNA, usually** around 202020 nucleotides in length. **Two** primers are used in **each** PCR reaction, and they are **designed** so that they flank the target region (region that should be copied). That is, they are given sequences that will make them **bind** to opposite strands of the template DNA, just at the edges of the region to be copied. The **primers bind** to the template by complementary base pairing. Template DNA: 5' TATCAGATCCATGGAGT...GAGTACTAGTCCTATGAGT 3' 3' ATAGTCTAGGTACCTCA...CTCATGATCAGGATACTCA 5' Primer 1: 5' CAGATCCATGG 3' Primer 2: Template DNA: 5' TATCAGATCCATGGAGT...GAGTACTAGTCCTATGAGT 3' 3' ATAGTCTAGGTACCTCA...CTCATGATCAGGATACTCA 5' Primer 1: 5' CAGATCCATGG 3' Primer 2: When the primers are **bound** to the template, they can be **extended** by the polymerase, and the **region** that **lies between them will get copied.**

The steps of PCR

The **key ingredients** of a PCR reaction are Taq polymerase, primers, template DNA, and nucleotides (DNA building blocks). The ingredients are assembled in a tube, along with **cofactors needed by the enzyme,** and are put through repeated cycles of heating and cooling that allow DNA to be synthesized. The basic steps are: Denaturation: Heat the reaction strongly to separate, or denature, the DNA strands. This provides?? single-

stranded template for the next step. Annealing: Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA. Extension: Raise the reaction temperatures so Taq polymerase extends the primers, synthesizing new strands of DNA. This cycle repeats 25- 35 times in a typical PCR reaction.

In order to study or detect individual genes or specific DNA regions or mutations of interest, it is often necessary to obtain a **large** quantity of nucleic acid for study. Rather than isolate a single copy of the target DNA from a large number of cells, it is often more useful to generate multiple copies of a target from a single molecule of DNA or mRNA, via an ***in vitro*** amplification method.

As the Polymerase Chain Reaction (PCR) is the **most** common DNA amplification method in molecular biology, NEB's product portfolio features a large selection of polymerases geared towards this powerful method. As the **first** company to sell *Taq* DNA Polymerase to the research market, the first to discover a PCR-stable, high-fidelity DNA polymerase, and the first to provide reagents for PCR performed in space**????**, NEB has a long history of developing reliable and convenient PCR tools. This commitment has continued with the recent development of One*Taq*® DNA Polymerase for robust routine PCR and Q5® High-Fidelity

DNA Polymerase for robust, ultra high-fidelity PCR (>280 X *Taq* fidelity). Both product lines have been developed to tolerate a variety of complex templates without experiencing a loss of performance on high-AT or high-GC targets. A variety of NEB polymerases, including One*Taq*, *Taq* and Q5, also benefit from novel aptamer-based hot start technology that does **not** require a separate activation step.

For experiments , where detection and quantification is required **instead** of isolation, quantitative PCR (qPCR) uses real-time fluorescence to measure the amount of a DNA target present at each cycle during a PCR. The most common methods of generating a fluorescent signal are by use of **hydrolysis** probes (e.g., TaqMan®), or a double-stranded DNA binding dye, (e.g., SYBR® Green). Ideally, qPCR master mixes should be evaluated for high reaction efficiency over a wide linear dynamic range, and low variation between repeated reactions across a broad variety of sample types. NEB's Luna® product line has been developed to simplify your qPCR reagent selection while accomplishing best-in-class performance.

Despite the ubiquitous nature of PCR and qPCR, it **may not be the best** option for all amplification needs. For point of care **and other** diagnostic applications, **sequence-specific isothermal amplification methods, that eliminate the need for thermocycling,** have been particularly useful. **Instead** of heat, these methods

typically **employ** a strand-displacing DNA polymerase, like *Bst* DNA Polymerase, Large Fragment, to separate duplex DNA. To address some of the **limitations of current isothermal** amplification techniques, NEB has developed the next generation *Bst, Bst* 2.0 and a WarmStart® version of this enhanced polymerase, which enables room temperature reaction setup, yet is fully active at temperatures greater than 50°C.

RNA **molecules** can also be detected and manipulated through amplification via the use of reverse transcriptases (RT), which are RNA-dependent DNA Polymerases. RTs polymerize a strand of DNA that is complimentary to the original RNA template and is referred to as cDNA. This cDNA can then be further amplified through PCR, qPCR or **isothermal** methods as outlined above **or** detected in a **single** reaction using **one**-step RT-qPCR or RT-LAMP.

Nucleic acid amplification is a foundational process in molecular biology and, as a testament to its utility, new protocols and modifications are being developed constantly.

Specific Applications

FAQs
Protocols
Tools & Resources
Publications
Legal Information

DNA Amplification, PCR & qPCR includes these areas of focus:

Isothermal Amplification
Loop-Mediated Isothermal Amplification
Whole Genome Amplification & Multiple Displacement Amplification
Strand Displacement Amplification & Nicking Enzyme Amplification Reaction
Helicase-dependent Amplification
Recombinase Polymerase Amplification and SIBA
Nucleic Acid Sequenced Based Amplification and Transcription Mediated Amplification

PCR
Routine PCR
High-Fidelity PCR

PCR & Reaction Cleanup

Polymerases for DNA Manipulation

qPCR & RT-qPCR
Dye-based qPCR & RT-qPCR
Probe-based qPCR & RT-qPCR

RT-PCR & cDNA Synthesis
cDNA Synthesis
RT-PCR
Site Directed Mutagenesis
Specialty PCR
Extraction-Free PCR
Hot Start PCR
Long Range PCR
Fast PCR
Multiplex PCR
Bisulfite Sequencing
Polymerases for NGS Library Preparation
Whole Genome Amplification
https://international.neb.com/applications/dna-amplification-pcr-and-qpcr

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Friend ,mentor of Kary Mullis = Thomas J White .
From 1978-1988, White was employed at the biotechnology firm **Cetus** Corporation where he held the positions of Vice President of Research and Associate Director of Research and Development. He worked on the discovery, research and development of human proteins as therapeutics, such as beta-interferon for the treatment of multiple sclerosis and interleukin-2 for renal cell carcinoma. He also directed the research and development

of products **using** the polymerase chain reaction (PCR) for multiple applications in basic research, molecular evolution, forensics and diagnostics. From 1989-2000 White worked for **Roche** Molecular Systems, a diagnostics division of **Hoffmann-La Roche.** As Senior Vice President of Research and Development, he was responsible for **Roche's** R&D on PCR-based tests for the medical **diagnosis** of genetic diseases, cancer (HPV, CML), infectious diseases (TB, CMV, STD's), for screening the blood supply for HIV, HCV and HBV, for **bone marrow trans**plantation (HLA), and for developing **new** applications of PCR for basic research, forensics and the **human genome project**. During that decade, Tom directed post-doctoral research fellows working on HIV evolution and transmission, and was a Visiting Scholar at UC Berkeley where he collaborated with Professor **John W. Taylor** on the molecular phylogenetics and population biology of human and plant fungal pathogens. From 2001-2011, White was Senior VP of R&D and Chief Scientific Officer at **Celera Corporation**. Celera Diagnostic's research involved the discovery of new genomic and expression biomarkers and the development and FDA registration of molecular diagnostic products for cystic fibrosis, Fragile-X, HIV drug resistance, and hepatitis C virus genotyping as well as laboratory developed (CLIA) tests for complex common diseases. Throughout his research career, White coedited four books and coauthored a hundred publications in peer-

reviewed scientific and medical journals. Dr. White retired in June 2011 and was the Regents' Lecturer at the University of California at Berkeley in 2012-13 where he is a member of the advisory boards of the Human Rights Center, the College of Natural Resources, the SAGE Scholars program, and a Trustee of the University of California Press Foundation. He is a scientific advisor to the Foundation for Innovative New Diagnostics (FIND) in Geneva and serves as a member of the board of Compassion & Choices, a nonprofit organization that provides support, education and advocacy for people with a terminal illness.

From 1978-1989, White was employed at the biotechnology firm Cetus Corporation where he held the positions of Vice President of Research and Associate Director of Research and Development. He worked on the discovery, research and development of human proteins and monoclonal antibodies as therapeutics, such as Betaseron for the treatment of **multiple sclerosis**, and on diagnostic tests **using** polymerase chain reaction (PCR) technology. From 1989-2000 he was Sr. Vice President of Research and Development at Roche Molecular Systems, a diagnostics division of Hoffmann-La Roche. He was responsible for Roche's R & D program on the AMPLICOR line of PCR- based tests and the COBAS instruments for the medical diagnosis of infectious disease, genetic disease and cancer, and in developing

new applications of PCR for basic research, forensics and the human genome project. From 2000-2011, White was Chief Scientific Officer at Celera. His work involved the discovery of new genotyping, expression and proteomic biomarkers and the development of molecular diagnostic products for complex common diseases (cardiovascular, autoimmune, cancer and neurological) as well as host response to infectious diseases. Since retiring, Dr. White was the Regents Lecturer at UC Berkeley in 2012-13 and has been an advisor to the Human Rights Center, the College of Natural Resources, a Trustee of the UC Press Foundation, the SAGE Scholars Program, the Foundation for Innovative New Diagnostics (FIND) in Geneva, and Compassion & Choices - a nonprofit organization that provides support, education and advocacy for people with a terminal illness.

https://en.wikipedia.org/wiki/History_of_polymerase_chain_reaction
https://en.wikipedia.org/wiki/Frederick_Sanger
https://en.wikipedia.org/wiki/PerkinElmer
https://en.wikipedia.org/wiki/Chiron_Corporation

Techniques used in recombinant DNA technology
V.SRIDEVI 25.11.2011."

Templates for PCR…. Dried blood , Semen stains . Vaginal swabs , Single hair , Fingernail scrapings, Egyptian mummies , Buccal Swab .

5 HISTORY Kary Mullis conceived the idea for the polymerase chain reaction in the spring of 1983; employee of Cetus Corporation, a biotechnology firm located near Berkeley, California.Kary Mullis conceived the idea for the polymerase chain reaction in the spring of 1983; employee of Cetus Corporation, a biotechnology firm located near Berkeley, California. Mullis & his assistant Fred Faloona tried to get it to work later in the year, & were soon joined by other Cetus scientists who saw the great potential of this method.Mullis & his assistant Fred Faloona tried to get it to work later in the year, & were soon joined by other Cetus scientists who saw the great potential of this method.

Problems with the error rate of Taq polymerase All DNA polymerases make mistake during DNA synthesis occasionally inserting in an incorrect nucleotide- DNA strand. **They are able to rectify these errors by reversing over the mistake and resynthesizing the correct sequence**.
This property is referred to as “proof reading”.

https://slideplayer.com/slide/10527933/

https://www.the-scientist.com/news/cetus-a-collision-course-with-failure-60308

It's not even probable, let alone scientifically proven, that HIV causes AIDS. If there is evidence that HIV causes AIDS, there should be scientific documents which either singly or collectively demonstrate that fact, at least with a high probability. There are no such documents.
*Spin*Magazine, Vol. 10 No.4, 1994.

Where is the research that says HIV is the cause of AIDS? There are 10,000 people in the world now who specialize in HIV. None has any interest in the possibility HIV doesn't cause AIDS because if it doesn't, their expertise is useless.

People keep asking me, "You mean you don't believe that HIV causes AIDS?" And I say, "Whether I believe it or not is irrelevant! I have no scientific evidence for it." I might believe in God, and He could have told me in a dream that HIV causes AIDS. But I wouldn't stand up in front of scientists and say, "I believe HIV causes AIDS because God told me." I'd say, "I have papers here in hand and experiments that have been done that can be demonstrated to others." It's not what somebody believes, it's experimental proof that counts. And those guys don't have that.
California Monthly, September 1994.

https://en.wikiquote.org/wiki/Kary_Mullis
https://www.brainyquote.com/authors/kary-mullis-quotes
https://www.quotetab.com/quotes/by-kary-mullis
https://en.wikipedia.org/wiki/Kary_Mullis

The Huw Christie Memorial Prize: $100,000 Reward for 'HIV' Offered by Alexander Russell 19th July 2002
"...infectious units, after all, are the only clinically relevant criteria for a viral pathogen." Peter Duesberg and Harvey Bialy (Nature, 375, 1995, p. 197)

I am still offering $100,000 Reward for the first person who can prove that 'HIV' exists via visual confirmation.
There is **no** evidence that 'HIV' is a sexually transmitted retrovirus and the current 2002 **UK Public Health Laboratory Service** figures clearly confirm this.
You simply **cannot** have a putative retrovirus that is permanently restricted for 20 years to the two originally identified risk group: gay men and drug addicts.
There is **no** heterosexual 'HIV/AIDS' epidemic in the UK, Europe and the USA and there **never** will.

In the Ukraine and Eastern Europe this is **not** an 'AIDS' epidemic but a recreational drug epidemic. It is the recreational drugs that are the activating factors; that are activating the endogenous material **wrongly** labelled 'HIV'. It is the chemicals in **cocaine** and other recreational

drugs that make people **test** 'HIV' positive and **not** the putative 'HIV'. **Cocaine** acts as an in vivo mitogen in exactly the same way that **other** plant derived substances have a mitogenic effect on cell-cultures in vitro. Indeed, experiments have shown that when cocaine is added to cell-cultures, the cells are **activated** and show a typical mitogenic response. **Cocaine** is the most obvious example but add to this the full repertoire of recreational drugs indulged in by many gay men and it is **no** wonder that their constantly activate cells permit the putative 'HIV' **tests** to dredge up something endogenous. Consider this: there is **not** one study which claims to show that any **animal** retrovirus is sexually transmitted so why should 'HIV' be the exception? There is **no** 'AIDS' epidemic in South Africa and there are **no** 'AIDS' graves. According to recent news reports citing national statistics, **life expectancy** in South Africa has increased by nine years during the period of time **known as** the AIDS epidemic, deaths in South Africa from **all** causes including 'AIDS' remain at less than 1% annually, infant mortality has not increased there in the past 20 years, and the country's population grows at a healthy 3% each year. What they have cynically remarketed and reclassified as 'AIDS' is in fact the global recreational drug epidemic and diseases which thrive in the Third World such as TB **and** malaria. Hans Gelderblom of Berlin's Robert **Koch** Institute co-authored the **first** paper in Virology, March 1997, showing **'purified** HIV' **to be 'purified** microvesicles'.

What was **assumed** to be 'purified HIV' **was in fact** "an excess of vesicles" - particles of cellular?/**tissue** proteins. The **hypothetical** 'HIV' **is in fact** a collection of endogenous microvesicles and cellular proteins (which also **never** seem to form particles - so **how** can they be infectious)? Cell-free viral 'HIV' particles have **never** ever been **visualised** in any freshly donated bodily fluid including semen, blood, etc. 'HIV' has **never** ever proven to be a sexually transmitted retrovirus. To date: **no** electron-micrograph image exists of isolated/purifed densely packed 'HIV' particles recovered **directly** from **fresh** samples of any bodily fluid. **The orthodoxy always comes up with cloned laboratory artefacts from which they adduce similar objects are to be** found plentifully in the wild. The **key fact to** remember is that cell-free infectious 'HIV' viral particles have **never**, repeat never, been recovered from fresh donor semen. It is homophobic nonsense to say 'HIV' is sexually transmitted via anal sex as well as scientifically totally **unproven**. 'HIV' is not an STD.

The rules demonstrating the existence of 'HIV' (and retroviruses in general) were **never** adhered to by those who **devised** them , **nor** were they ever **validated**. No particle of 'HIV' has ever been obtained pure, free of contaminants; nor has a complete piece of 'HIV RNA' (or the transcribed DNA) ever been proved to exist. The immunological-stressors of the 'gay life style'

(recreational drug use, antibiotics, **flu** jabs, alcoholism, untreated STDs, etc) can **make** many gay men test 'HIV' **positive**. **All 'HIV' testing kits come with the warning that they must not and cannot be used as diagnostic tools to prove 'HIV' infection.**

So confident am I that no such electron-micrograph evidence for the existence of 'HIV' can be produced by adhering strictly to the Etienne de Harven methodology, I am prepared to offer the sum of $100,000 to the first person to submit just such a micrograph, prepared under **stringent** laboratory conditions.
I do **not** want **'markers' for 'viral activity'** which are at **very best, inaccurate.** I want visual evidence of myriad active, infectious viral particles, clearly morphologically defined , recovered from a fresh sample of bodily fluid, **unadulterated** with any other kinds of cells: i.e: CEM,H9 cancer cells.

As Peter Duesberg and Harvey Bialy stated in Nature: "...infectious units, after all, are the only clinically relevant criteria for a viral pathogen." (Nature, 375, 1995, p. 197)

Once again, to paraphrase Peter Duesberg, an **alleged** 'virus' which is not doing anything , cannot be 'causing' anything. The rules for attempting to isolate the putative 'HIV' via the Etienne de **Harven methodology** are: 1.

Only plasma centrifuged from **fresh** whole blood may be used in the experiment.No material derived from **cultured** cells will be considered, to rule out 'viral particles' which may be merely **cultural artefacts**. 2. The donor blood/plasma must be taken **from** a person/persons with a **recent 'high-viral load'** test result, and evidence for the date and result of the test (the number of 'HIV'- RNA's alleged) must be submitted, obviously with the name of the person/persons deleted to preserve donor confidentiality. 3. The donor must **not** be in receipt of protease inhibitors, AZT or any 'antiviral drugs'. 4. **Only cold heparinised Ringer's solution may be used to dilute the plasma** 1/1 (i.e. 50%). 5. The diluted plasma shall be first filtered by aspiration-filtration, through a 0.6 millipore membrane. The resulting filtrate #1 will then be filtered again, this time using a 0.22 millipore membrane and filtrate #2 will be submitted to ultracentrifugation. 6. **Centrifugation** at 30,000 g for two hours will be used to prepare a pellet, likely to be extremely small. This pellet will be fixed with **glutaraldehyde** and **osmium**, then carefully detached and embedded in epoxy resins following routine EM procedures.
7. The electronmicrograph shall be at least 19,500 x magnification, and **must** resemble that published in Fig.1 of this article for particle size and shape, **but** with one notable and important variation. 'HIV' has been **deemed** to be a lentivirus, possessing a dense core of truncated conical shape. An ultrathin slice of randomly packed

lentiviruses must inevitably show a number of particles bisected to show this core lengthwise, as well as end-on, with a resultant apparent mixture of round and 'rod-shaped' dense cores. Any micrograph which does **not** clearly show this feature will be deemed **not** to represent the lentivirus 'HIV'. 8. This challenge is **open to any** qualified scientists, or microbiology students/lab technicians with the necessary lab skills and facilities to carry out the work. //Emeritus Professor of Pathology, University of Toronto. He worked in electron microscopy primarily on the ultrastructure of retroviruses throughout his professional career of 25 years at the **Sloan Kettering** Institute in New York, and 13 years at the University of Toronto.

RETROVIRUS: THE MEMORIES OF AN ELECTRONIC MICROSCOPIST.

Etienne de Harven. For an electron microscopist who has devoted almost his **entire** research career to the study of **retroviruses** associated with mouse leukemia and who has followed with great attention the **hypothetical** impact that such research could have had in the study of cancers in man, it was **predictable** that current AIDS research would go in the wrong direction. How was it predictable? The memories collected below are intended to make him understand. The **importance** of electron microscopy in the development of modern cell biology, between 1945

and 1965, is unanimously recognized??. The relationships which unite cellular structures and functions could, without doubt, never have been well understood **without** the use of the very great separating power of the electron microscope (ME)???. What may **not** be as widely appreciated, however, is the role that virology has played in the study of **cellular??** ultrastructures. Historically, when in 1931 **Rüdenberg** (1) applied for a patent for his **invention** of the electron microscope, his main hope was to be able to **visualize** the poliomyelitis virus! And during the **second** world war, when electron microscopes began to become **accessible** to biologists, priority was given to efforts to discover viral particles associated with the cancer cells of certain laboratory animals. This is how **Albert Claude,** working at the **Rockefeller** Institute in New York succeeded in demonstrating the Rous sarcoma virus in chicken fibroblasts (2). And a few years later, **Keith Porter** and his associates had similar success in obtaining images of the "milk factor" in mouse breast adenocarcinoma cells (3). The viral **origin** of Rous sarcoma in hens and mammary tumors in mice appeared to be well established**???** by microbiological experiments based on techniques of **ultrafiltration** and performed **well before the first** electron microscopy images were published. And yet, **direct** observation of viral particles in these experimental tumors **gave??** an extraordinary impetus (today, we might say excessive!)

In the search for viruses in oncology. The **viral** origin of certain cancers in mice and hens had been clearly demonstrated by **ultrafiltration** experiments which made it possible to approximately **assess the diameter** of the viral particles. The electron microscopists therefore knew in advance the **size** of the particles they had to try to identify, this dimension being frequently about 100 nm. This facilitated the **initial** identification of so-called "oncogenic" viruses by electron microscopy, although it **later** became clear that **countless** microvesicles or particulate elements of **normal** cells were approximately the **same** diameter. The discovery by **Charlotte Friend,** working at the **Sloan Kettering** Institute of New York, of a mouse erythroleukemia transmitted by acellular filtrates illustrates well the research methods used around the years **1955**. In addition, as it turns out that I started to working in the laboratory of Dr. Charlotte Friend at that time, the principles that we applied to our research are particularly familiar to me. For electron microscopy, we gave priority to two kinds of samples: 1) different tissues from leukemic "Swiss" mice (spleen, lymph nodes, thymus and bone marrow), and 2) pellets obtained by ultracentrifugation of filtrates. acellular leukemia tissues, filtrates which we knew to be they effectively transmitted the disease by injection to adult "Swiss" mice, or to mice of the DBA / 2 strain. We knew, through **filtration** experiments, that the activity (ie the power to transmit leukemia) **disappeared** when we used filters whose pore

diameter was less than 200 nm. The classical theories of **ultrafiltration** therefore allowed us to predict that the infectious particles should have a diameter close to 100 nm. Studying leukemia tissue under the microscope technique using ultrafine sections frequently revealed particles of this diameter, closely associated with various cells. The particles appeared to be surrounded by a simple membrane and had in their center a nucleus, or electron dense nucleoid. Their ultrastructure was characteristic and their diameter remarkably constant. To our knowledge, such particles did not resemble any known components of normal cells. However, they resembled particles identified by other authors in several "filterable" experimental tumors and **classified** by W. Bernhard as "type C" particles (4). In addition, we observed identical particles in pellets prepared by ultracentrifugation of acellular filtrates capable of transmitting the disease to susceptible mice. It is on the basis of these data that we hypothesized that these particles indeed represented the "oncogenic" virus etiologically linked to Friend's erythroleukemia (5). We were however surprised to observe the **virus** in close association with cells which were apparently not involved in the leukemic process, such as megakaryocytes of the bone marrow, for example. These electron microscope studies **had also shown,** from the start, that **not** all electron dense particles with a diameter close to 100 nm were **viruses**, and that a rigorous **ultrastructural** analysis was essential to distinguish from a appropriately **viruses**

and "virus-like particles". Fortunately, our electron microscopy studies quickly added important data for the identification of oncogenic RNA viruses. It appeared that these viruses were formed on the cell **surface**, the cell membrane of **infected** cells **directly contributing to** the formation of the future viral envelope by a series of stages to which we have given the name of budding phenomenon ("budding") (6). Viruses are released into the intercellular **spaces** by this budding process. Identification of viruses in this group has therefore become more rigorous, with observation of budding particles now required. This likely **eliminated** thousands of images **from " medical literature!** In addition, the observation of particles in the process of budding at the level of cell surfaces allowed us to identify the infected cells, one by one, and to conclude that these are perfectly viable, in the **absence** of any sign of lysis. infected cells, infection with viruses of this type therefore having no cytolytic effect. In addition, the viruses were **clearly identified**??? in cells undergoing mitotic division (7). Since, obviously, human experimentation is unacceptable, the possible observation in human cancer cells of particles resembling those described in experimental tumors could have been of great interest, although not sufficient to draw the slightest conclusion. In the **1960s**, many laboratories around the world, using the **latest** refinements in electron microscopy techniques, attempted to demonstrate this. At that time, that is to say well **before** the emergence of

molecular biology, electron microscopy was, **without** a doubt, the method of choice to try to identify viruses in cell samples. The crucial role of electron microscopy in virology was to Continuing our research on the Friend's leukemia virus, and encouraged by Dr. J. Beard of Duke University (Durham, North Carolina) who had considerable experience in avian leukosis, we focused our efforts on demonstrating, by electron microscopy, of viremia (presence of virus in circulating blood) in leukemia mice. The most effective initial step in purifying the avian leukosis virus was to start not with tissue but with blood plasma from leukemia chickens. This was of the **utmost** importance for us because, in fact, we did not obtain very satisfactory results, in terms of **purification** of the Friend virus, when we used homogenates of leukemic tissues such as the spleen or the lymph nodes. . We have therefore developed a **very simple purification** method from the blood plasma of mice, and based on a **double** ultrafiltration on "Millipore" membranes. A diluted sample of plasma, 10 ml from the bleeding of approximately 25 leukemic mice, was first clarified by aspiration through a filter of porosity 0.65 μm; the first filtrate was then subjected to a **second** filtration, this time using a 0.22 μm filter. The second filtrate was then centrifuged for 120 minutes, at 30,000g. The result was an extremely small, barely visible **centrifugation** pellet, which could be prepared for electron microscopy. The ultra-thin sections of these caps revealed the presence of a

remarkable population of typical and well-preserved viruses, packed against each other, and with very little contamination by **cellular** debris (9). This was our approach to the demonstration of viremia in 1965 ... And during this time, many laboratories of electron microscopy centered on oncology (that of Dr. W. Bernhard, in Villejuif, in France, of Dr. AJ Dalton, at the National Cancer Institute, Bethesda, Maryland, of Dr. L. Dmochowski, at MD Andrerson, Houston, Texas, and ours, at the Sloan Kettering Institute in New York), were investing a huge part of their research time in trying to demonstrate viral particles associated with cancer in humans . "Virus-like particles" have occasionally been observed, but have **not** convinced anyone! Typical viral particles have **never** been conclusively demonstrated. And this was in stark **contrast** to the ease with which one could demonstrate, by electron microscopy, viruses in several leukemias and cancers in mice and chickens. Very few publications have been devoted to these **negative** results on cancers and leukemias in humans. And yet, Haguenau, in 1959 (10), underlined the **difficulty** that there was in identifying the smallest viral particle in a large series of breast cancer. **Bernhard** and **Leplus, in 1964 (11), in a book devoted to** the study of a large number of cases of Hodgkin's disease, lymphosarcomas, lymphoid leukemias and metastatic diseases, **failed** to identify viral **particles** associated with these various pathological conditions. At the **Sloan Kettering** Institute

in New York, I decided in 1965 to **stop** all studies under the electron microscope of leukemia and lymphoma cases for the presence of viral particles, **after several years of entirely negative research.** I reported on this decision at a conference on "Methodological Approaches to the Study of Leukemias" held in Philadelphia at the Wistar Institute in 1965 (12). Of mice and Men.... The publication of all these **negative** results did **not succeed in discouraging fanatic virus hunters!** An explanation for these **negative** results should therefore be found elsewhere! Perhaps the **technique** of ultra-fine cuts in electron microscopy was **not** the best? (although it did so well in mice!). Preparing ultra-thin cuts was time consuming and skillful! Who still had time for this when research funding became difficult to obtain and when the giants of the **pharmaceutical** industry began to offer attractive contracts for quick responses? Why not try the negative **coloring** technique? It's very easy, and it goes very fast! And, after all, this technique had given remarkable results in the study of **viruses devoid** of envelope such as the adenoviruses and the polyome. The results were absolutely **disastrous** with regard to RNA viruses associated with tumors (**not yet called** retroviruses ...), because these viruses are **fragile** and are completely **deformed** by air drying which is inevitably part **negative** staining technique; seen by this technique, viruses appear as particles with a **long tail**! Unfortunately, many cellular debris and many microvesicles, after air

drying for negative staining, **also form profiles of particles with a tail.** The temptation to **interpret** all the "particles with tail" how oncogenic RNA viruses was , was great and appeared to be an extraordinary boon for virus **hunters**! And yet, we had clearly demonstrated that the "viruses with a tail" **were artifacts due to the negative staining technique, artifacts which could be easily avoided by an appropriate control of the osmotic power and by the fixation with osmic acid preceding the negative staining** (13), **or** by the critical point drying technique (14). The enormous **confusion** created by publications on "tailed particles" has done considerable damage to the credibility**???** given to electron microscopy in the search for viruses associated with cancers. We were looking for "tailed particles" in cow's milk and human milk, and Sol Spiegelman spoke eloquently about the **risks** of **breastfeeding** ... An important discovery, which had **nothing** whatsoever to do with electron microscopy, completely reoriented ideas concerning the possible mode of action of oncogenic RNA viruses. It was the discovery by **Temin** and **Baltimore**, in 1970, **of the enzyme** "reverse transcriptase" (reverse transcriptase, RT). We were apparently beginning to **guess** how it was possible for oncogenic RNA viruses to modify the genome of infected cells. In addition, these viruses remained good candidates as possible "oncogenic" factors because they were well recognized as **NON** cytolytic (ie they do not kill the cells

they infect). As a **result**, the oncogenic RNA viruses were **renamed**. We decided to call them "**Retrovirus**" (**retro, for RT**). The direction of research efforts changed considerably after the discovery of reverse transcriptase (RT), that is, after **1970**. In fact, all the methods that had dominated the study of viral oncology from 1950 until 'in 1970 were gradually **replaced** by a very exclusive fashion of molecular biology methods. I observed this evolution rather from the outside, because, in my opinion electron microscopy was **no longer** the main method that would allow us to advance in the study of the **hypothetical** relationships that would exist between retroviruses and cancers in man. The years 1970-1980 were **dominated** by a **series** of ideas whose scientific value would never have been accepted 10 or 20 years ago. For example: 1. It **became acceptable** to state that, when viruses could not be identified by electron microscopy, other methods of a biochemical or immunological nature, **supposedly** capable of identifying viral "**markers**", were sufficient to demonstrate the viral infection of the cells studied. These "**markers**" could be an enzyme (RT), an antigen, various proteins, or certain RNA sequences. The fact of **never** having seen viral particles under the microscope was **very conveniently explained by** the integration of the viral genome **into** the chromosomes of the **allegedly** infected cells. **Accepting such an interpretation implied complete ignorance of everything** we had learned during the study of experimental cancers in laboratory animals. It

must be recognized, however, that in these experimental **models**, electron microscopy **only** observed**???** the **final** stage of viral multiplication, the **initial** stages consisting of a **series** of molecular events which **escape** completely to ultrastructural images. And yet, in all classical experimental systems such as avian or murine leukosis, the terminal phases of viral replication (budding) were **always** observed **and** considered essential for the spread of viral infection from a cell to cell. 2. Another short circuit with disastrous consequences was this very naive **notion** that all biological materials sedimenting on a sucrose gradient at a density of 1.16 g / ml **were** retroviral in nature! Undoubtedly, well characterized retroviruses , sediment around this density**???**. But this does **not** imply that everything that sediments at this density is retroviral in nature! In the 1960s, fellow biochemists often asked me to look (with an electron microscope) at certain "bands" sedimenting at density 1.16: "Look at this, it forms a clear band at 1.16, it must be pure retrovirus!". The **ultracentrifugation** pellets obtained from these famous "1.16 bands", studied in **fine** section by electron microscopy, recognized a wide variety of microvesicles and cellular debris, but **not** a single retrovirus! However, this 1.16 density sedimentation method **is still used to identify so-called viral "markers"!** How distressing to think that an adequate control , under the electron microscope of these famous "1.16 bands" (which takes about 2 days and costs a few hundred dollars only) could

have avoided these dangerous interpretations of so-called "retroviral markers" on which huge research budgets have been wasted miserably ... 3. The **isolation** of viruses **from** the supernatant of infected cell cultures raises **other** questions. We all remember the discovery by Epstein (15) in **1964** of the EB virus in cell cultures obtained from African cases of Burkitt's lymphoma. This discovery was **based** on electron microscopy and this virus was immediately and correctly**???** classified as a member of the herpes group. To **identify** this virus in cells in culture it was **preferable** to observe cells in the process of **degeneration**, because, obviously, this virus had indeed marked cytolytic effect. On the contrary, cells infected with **retro**viruses maintain excellent viability**??**, which makes it possible to **isolate** these viruses from the supernatant of cultures, 4. With regard to scientific research policy, it was clear that research on so-called oncogenic viruses was **dominated by the retroviral hypothesis.** Almost all federal appropriations took this **same** direction, especially since the very naive idea prevailed that , the success of scientific research was above all ... a question of **big money!** The scale of the credits granted has enabled the **creation** of a considerable retroviral research apparatus, with many new jobs. Unfortunately, intellectual freedom to think of other directions of cancer research was going to diminish, especially as the **giants** of the pharmaceutical industry began to offer almost irresistible contracts, **strongly**

polarized exclusively on retroviral research ... The highest **priority** was to demonstrate, at any cost, that **retroviruses** had something to do with the origin of cancer in humans, a **hypothesis** which did not, however, **not** received any experimental support during all the 1960s and 1970s. Such a poorly directed research effort would perhaps have had little consequence as long as public health was **not** directly involved. Unfortunately, the onset of AIDS, the acquired immunodeficiency syndrome, in 1981, quickly transformed what could have been just a regrettable academic misstep into a real tragedy. What happened after 1981 is so well known to readers of "Reappraising Aids" that I hesitate to elaborate on it in detail. The events leading up to the current crisis have been summed up and analyzed most convincingly by Peter Duesberg (16). I must admit that I read Duesberg's (1996) book with the greatest attention, although essentially unsurprisingly, since retroviral research had **dangerously set the stage for "Impure Science"** in the 1970s (17). Shortly after the first cases of what we started to call the "Gay related immune deficiency" (GRID) were described by **Michael Gotlieb** it was clear to all observers that **Gallo** and his associates were going to devote themselves headlong to the new a syndrome that seemed to them an unexpected opportunity to try to justify the considerable federal budgets they had spent on the study of retroviruses for the past 10 years. Because it must be remembered that, in 1980, the scientific community

became more and more impatient at the complete lack of results of the "War against Cancer" based on the hunt for viruses. The minor episode of HTLV-1 was far from enough to allay fears of grossly wasting federal research funds. And the fact that the **new** syndrome, quickly **renamed** "AIDS", had very little to do with cancer did not embarrass **Gallo** more than that. The frequent association of the syndrome **with** Kaposi's sarcoma also made it possible to hide the difference in the eyes of the general public. Dominated by the media, pressure groups and the interests of several pharmaceutical companies, official AIDS research sought to control the disease, having lost all contact with free scientific thought and traditional medical research ("peer reviewed "). The HIV = AIDS **hypothesis**, which had **not** yet been demonstrated, drained 100% of research funds, while all the other hypotheses were ignored. Both the general public and the medical community **have been led to believe that the presence of antibodies in the circulating blood makes it possible to diagnose a progressive disease, that Koch's postulates have gone out of fashion,** that 90% of cases of an infectious disease can be observed in **male** patients, And to further confirm the official **hypothesis**, we found it preferable to forget that it had been known for decades that **heroin** addicts were exposed to serious immunodeficiencies, that the inhalation of **nitrite** has many toxic effects. , that the **extreme** toxicity of **AZT** has been known for 20 years,

that of **all known retro**viruses **none** has a cytolytic effect, etc, etc ... In addition, to allow the "AIDS business" to develop profitably, research on any dissident **hypothesis** (that is to say non-HIV) was carefully **undermined** by very tight control of research funds as well as by the 'extreme difficulty which quickly appeared to publish, anywhere, the **least** dissenting opinion ... Around the years 1985, I considered adding to my research programs the study with the electron microscope of patients suffering from AIDS. Unfortunately, the media had already by then orchestrated the panic of an **epidemic worse than the plague,** and my assistants quickly made me understand that if I insisted in this direction they would all leave the lab! The "HIV positive" test was still considered to be diagnostic **reliable** at the time. Since then, we have understood, through the work of Papadopulos and the Perth group in Australia, **that this test is far from being specific** (18)! Since I retired to France, I have taken every opportunity to speak as freely as possible about the issues raised in this article. I am proud to be a member of the California-based "Group for the **re-**evaluation of the HIV-AIDS hypothesis". I sincerely hope that the various activities of this group will provoke the initiation of new research on the causes of AIDS, for the greatest interest of the patients, and for the revival of scientific integrity in medical research!

REFERENCES: 1. Rüdenberg, R. (1932). Elektronenmikroskop (Electron microscope). Naturwissenschaften 20, 522. 2. Claude, A. (1947-1948). Studies on cells: morphology, chemical constitution, and distribution of biochemical functions. The Harvey Lectures, Series XLIII, pp 121-164. 3. Porter KR & Thompson, HP (1948). A particulate body associated with epithelial cells cultured from mammary carcinoma of mice of a milk factor strain. J. Exp. Med., 88: 15-85. 4. Bernhard, W (1960). The detection and study of tumor viruses with the electron microscope. Cancer Res. 20: 712-727. 5. de Harven E. & Friend C. (1958). Electron microscope study of a cell-free induced leukemia of the mouse: a preliminary report. J. Biophys. Biochem. Cytol. 4: 151-156. 6. de Harven E. & Friend C. (1960). Further electron microscope studies of a mouse leukemia induced by cell-free filtrates. J. Biophys. Biochem. Cytol. 7: 747-752. 7. de Harven, E. (1962). Ultrastructural studies on three different types of mouse leukemia; a review. In "Tumors induced by viruses" pp. 183-206, Academic Press, Inc. New York. 8. Lwoff, A, Horne, R & Tournier, P (1962). Cold Spring Harbor Symposium on Quantitative Biology 27:51. 9. Friend, C & de Harven, E (1965). A new method for purifying a murine leukemia virus. Fed. Proc. 24, No. 2. And: de Harven, E. (1965). Viremia in Friend murine leukemia: the electron microscope approach to the problem. Pathology-Biology 13 (3-4): 125-134. 10. Haguenau, F. (1959). Breast cancer in

women. Comparative study with the electron microscope and the optical microscope. Bull. Assoc. Franc. Study of Cancer, 46: 177-211. 11. Bernhard, W. & Leplus, R. (1964). In "Fine structure of the normal and malignant human lymph node". Pergamon Press, ed., Oxford. 12. de Harven, E. (1965). Remarks on Viruses, Leukemia and Electron Microscopy. In: "Methodological Approaches to the study of leukemias". Defendi, V. edit .; The Wistar Institute Press, Philadelphia, publ., Pp. 147-156. 13. de Harven, E & Friend, C (1964). Structure of virus particles partially purified from the blood of leukemic mice. Virology 23: 119-124. 14. de Harven, E, Beju, D, Evenson, DP et al. (1973). Structure of critical point dried oncornaviruses. 15. Epstein, MA, Achong, BG & Barr, YM (1964). Virus particles in cultured lymphoblasts from Burkitt's Lymphoma. Lancet 1: 702-703. 16. Duesberg, P (1966). "Inventing the AIDS Virus", Regnery Publishing, Inc., Washington DC. 17. Epstein, S (1996). **"Impure Science; AIDS, Activism, and the Politics of Knowledge".** University of California Press, publ., Berkeley CA. 18. Papadopulos-Eleopulos, E, Turner, VF & Papadimitriou JM (1993). Is a positive Western blot proof of HIV infection? Bio / Technology 11: 696-702.
www.sidasante.com/edh/edhsouv.htm
http://www.sidasante.com/science/sciindex.htm
The Top 100 inconsistencies AIDS Scientists by John Kirkham January 16, 2003.

www.sidasante.com/science/top_100_incoherences_sida.htm

HIV - Artifact or reality A virologist, **Dr. Stefan Lanka,** asks some very relevant questions: why, has HIV **never** been isolated? Is it possible to detect **antibodies** to a virus that cannot be isolated? Does HIV Really Exist? By answering these questions, Stefan Lanka demonstrates why there is **no** scientific basis for AIDS research "Even repeated over and over again, an error does not become truth.

For years, it has been officially stated that it is the human immunodeficiency virus, HIV, that causes AIDS. This virus is **believed** to be found in many bodily secretions; its transmission, which would be done particularly by the sperm **and** blood vectors, would trigger a **slow** and inexorable process of intoxication, **until** the declaration of AIDS; whose outcome would be fatal. However, if it is capable of infecting another cell, HIV must necessarily be **identifiable** as an entity separated at one point or another during its life cycle. The general public is kept in the dark about a set of major facts: **first**, there is **no** test for HIV that is effective; **second**, the **definition** of "HIV positive" is very rough. Each organization that has looked at HIV has given a **different** definition. Each type of screening test is based on a **different** definition. Even the laboratories preparing these tests are not sure of the

definition of seropositivity. "... These techniques are **not** standardized; and the consequences of variations in amplitude found **between** laboratories have **not** been evaluated. The noisy controversies about the attribution of the "**discovery**" of the virus (2), have obscured **the real question: does the virus exist - yes or no?**
It is necessary to bring together a President and a Prime Minister (3) to attribute a scientific discovery and public opinion is so impressed that it is convinced that the object of their negotiations must indeed exist. In 1993, a group of Australian researchers from Perth managed to publish a study on HIV testing. (4) Since then, anyone who can read , can understand that , **no** test can work , since HIV does not work. has **never** been isolated and that its very existence has **not** been proven. Since the media as well as the researchers have **ignored** any criticism of the thesis "HIV = AIDS", and especially the **fundamental** question of the existence of this virus, it is time to proceed to a **new evaluation of the whole of the thesis** HIV / AIDS. Going back to the **sources** of virological studies on HIV by **clearly exposing the entire history of** HIV, allows us to realize that HIV, the object of all "cutting-edge" research in modern medicine, does **not** exist! (5) A little elementary virology . **Viruses can be briefly described as** a set of genetic information contained in an envelope of proteins. Viruses can **only** reproduce if they infect a host cell appropriate to their species **and** they manage to take possession of the chemical mechanisms of this cell.

The proteins that surround viruses are **specific** to each species of virus. These proteins have the function of securely wrapping **and** transporting genetic information; their **composition** gives a specific form to each viral particle. **This is what is generally known about viruses.** The existence of particles that **resemble** viruses **but are not** viruses is a fact of much lesser notoriety. These particles, which respond to the **vague** designation of "virus-like" are however far from rare: they are found in all **placentas** and they are **very common** in **artificial** media of cell cultures. These particles have greatly contributed to **disturbing** research on AIDS, because they are particles of this type which have **been** called the HIV virus. To date, **none** of these particles has been sufficiently **defined**, **nor** has their existence been proven as a **separate** entity which could reasonably be called a virus. **Why** did people start believing in the viral origin of AIDS? In classical **theory**, DNA encodes the genetic material of heredity; it is then **transcribed** into RNA messengers (mRNA) which, **in turn,** determine the assembly of amino acids that build the proteins of all living things.In 1970,an enzyme (ie a biological **catalyst**) was discovered in certain cells, which proved capable of **converting** an RNA molecule into DNA. It was a revolutionary discovery, which **overturned** one of the **fundamental** dogmas of molecular genetics, namely that the flow of information **always** flows in **one** direction. It had always been thought **until then** that DNA was

transcribed into RNA messengers and that the **reverse** movement of RNA to DNA was strictly **impossible**. The enzyme capable of this reverse transfer was called " An **old error:** "cancer is of viral origin" .It was **believed** that this new enzyme, the reverse transcriptase, **was the marker of a virus,** because the cells in which it was discovered were cancer cells. (7) However, it was **believed** that the cancer affecting them was of viral origin. Another fact that reinforced the thesis of the viral **origin** of cancer (8): the nucleic acid, in its RNA form, could be converted into DNA by **inversion** transcriptase, thus creating a mechanism for inserting , anywhere, on the chromosomes of cells, the nucleic acid of the **virus**. (9) These "**new**" viruses were **baptized** retroviruses, (10) and one was persuaded that The idea that these **supposed** viruses could cause cancer immediately fascinated the scientific world; but **later** studies proved its falsity (11) and other explanations were sought. (12) Indeed, the theory of the viral origin of cancer proved incapable of predicting or explaining the lightning increase in the number of cancer cases, especially since it was **not** possible to demonstrate the **contagiousness** of the cancer, or the possibility of opposing a vaccine. (13) Characteristically, homosexuals, prostitutes and blacks were accused of transmitting these viruses themselves - saying carcinogenic. The **same** phenomenon happened again thirteen years **later** with AIDS. (14) It was hastily **assumed** that each **inverting** transcriptase activity was a

foolproof sign of the presence of retroviruses. This turned out to be a serious **error**, since the presence of this enzyme was **later** discovered in **all** living matter, thus demonstrating that the activity of **reverse** transcriptase is **independent** of the presence of **retroviruses**. (15) Repetitive elements …. Subsequently, research has shown that at least 10% of mammalian DNA is composed of repetitive sequences called "**nonsenses** genes" (meaningless genes); some parts of these genes were **nevertheless** described as **retrovirus** genes. These **repetitive** elements exist by the hundreds, if not by the thousands. Some may **even** reproduce independently , and jump in and between chromosomes; this is **why** they were given the **new** name of **retrotransposons**. We succeed in migrating them to the laboratory. When such displacement occurs, there is always **inverting** transcriptase activity; which **reinforces** the certainty that the activity of this enzyme has **nothing** to do with **retroviruses** as such. (16) VAL, VLTH-III, HIV and the others … All this was **already** well known in 1983 and it is **unlikely** that **Françoise Barré-Sinoussi** (who was part of the **Montagnier** team), as well as the **Gallo** team, **could claim** in 1984, having discovered a **new** virus, **when** they had **only** shown a case of reverse transcriptase activity and published photographs of cell particles **without proving** that they were viruses. These **two** teams were **unable** to **isolate** the "viruses" any more than they demonstrated that they **caused** the inversion transcriptase

activity **or** the tissue abnormalities from which they originated. (17) Their **conclusion** was described elsewhere: **"The role of the virus in the etiology of AIDS remains to be demonstrated".** But **what** exactly is a **new** virus? The isolation **and** purification of a **new** virus is clear: unlike cells?, viruses of the **same** species are always the **same** size and can be **easily** separated from other components of the cell using standardized techniques. One of the **control** experiments consists in trying to **isolate** with possible **non**-contaminated materials , in the **same** way as with infected materials. In this case, **no** insulation should be possible.

To definitively identify a virus, the first and simplest step is to photograph the isolated particles using an electron microscope. These particles must then have the same appearance as the particles observed in cells, bodily secretions or cell cultures. They should be able to distinguish them from **other** cell particles that **look like** viruses. The proteins that make up the protein envelope of said virus **must** be identified separately from one another and photographed. This set of operations makes it possible to establish a characteristic diagram of the **species** of virus studied. The **same** process of identification **and** isolation of RNA and DNA from the virus must be observed. There is **no** evidence of the existence of HIV .These basic demonstrations have **never** been done with regard to HIV. **No** photographs of the isolated HIV particles, its proteins or its nucleic acid have

been published. **None** of the control experiments mentioned above have been published. The photographs that have in fact been shown **represent** virus-like particles in cell cultures, but **no** photos of the isolated virus have been published, let alone a structure installed in a human body that would have the form attributed to HIV. What the whole world has been able to contemplate are **models** of HIV with dish aerial receptors which we are **told are receptors** with which the virus attaches to cells. The existence of HIV is **assumed** by deduction from the existence of an **antibody** test. But **how** could this test work when the existence of the virus itself has **never** been demonstrated and this virus has **never** been obtained **without** contaminant; that remains a mystery. The AIDS test ..We should remember that the AIDS test is **supposed** to detect antibodies produced by the immune system in response to infection by the virus. This is commonly done **as** follows: the rows of proteins from the virus**??** are placed on plastic racks and the blood serum to be tested is spread on each row. **If** antibodies are present, they attach to proteins, a phenomenon made visible using sophisticated **staining** methods. But since **no** viral protein free of contaminants exists, it is **never** possible to know the **real** nature of these antibodies that attach to proteins. This is the heart of the problem for all tests **supposed** to detect HIV: there is an **inability** to isolate a viral entity and to obtain proteins from it which are **not** contaminated by the proteins of the cells in which

the **supposed** virus is **supposed** to be developed. Using **indeterminate** antibodies to demonstrate the existence of a virus that has **never** been isolated is simply a **vicious circle**. **Why HIV tests cannot be functional.** It is therefore completely illogical to argue that a positive test result is due to prior exposure to the virus. (19) Because it uses various **misidentified** proteins, **each** manufacturer of the test kits applies their own **arbitrarily** chosen criteria , so **no two kits give the same result**. It does **not** matter that this or that academic committee sets standards against which , one test is said to be effective and another is not. This is just **one** way around the crucial problem: **what do the antibodies detected by** AIDS tests **react to?** The existence of "second" or "third" generation tests does **not** solve the problem. **Hiding the true identity of these proteins or advocating the use of two types of tests -** improperly dubbed "research" and "confirmation" to give a **false impression of security - does nothing to resolve the difficulty.** The ELISA test is used to sample the antibodies and then the more specific Western Blot is used as "confirmation". Nothing more tragically demonstrates the dilemma in which we are trapped than this extract from the leaflet which **accompanies** the test kit: "The test intended to reveal the existence of antibodies to the virus associated with AIDS **is not a diagnosis** of AIDS or AIDS-like illness. A negative result does **not** exclude the possibility of contact or infection with an AIDS-associated virus. A positive result does **not**

prove that the person tested has contracted AIDS or is about to declare AIDS or that she will contract it. " (20) We don't make them say!

Direct evidence of HIV ..Some researchers working on HIV have tried to work around the problem by showing something called "direct" evidence of the existence of the virus. The maneuver consisted in **arbitrarily** selecting a protein of a given size which **happen** to coincide with the HIV **models**. The **falsehood** of such "proof" became obvious when it was discovered that the protein in question was of ... **human origin**! (21) This is how genetic information about HIV **was made** ... **Despite** this deplorable state of affairs, the majority of researchers working on AIDS **still believe in** the authenticity of HIV **because** one of its supposed genetic sequences has already been published. In **addition**, there are now genetic procedures which, **unlike** anti-body tests, try to identify the presence of HIV more or less **immediately without** waiting for the weeks necessary for the formation of anti-bodies. The fact that genetic tests (PCR) (22) do **not** give the same result , **as** tests for antibodies is simply **overlooked**. Since **no** virus has been isolated, it follows that it has also **not** been possible to isolate the nucleic acid. Complicated processes in the scientific literature have been described that produce **something** called HIV nucleic acid. (23) 22: The little confidence placed in the validity of these tests is evident when we read the **warnings** in the package leaflet which accompany one of

them: "The Amplicor HIV-1 PCR test was tested **only** at using **whole** blood specimens. Its performance in the presence of **other** specimens has **not** been evaluated and may provide **false** positive or false negative results ... Detection of HIV-1 may **depend** on the **amount** of **proviral** DNA in the specimen. **Methods** of specimen collection **and** patient-specific factors such as age, health status, risk factors, etc. may **affect** the performance of the test. **As with any** diagnostic test, the **results** of Amplicor HIV-1 should be **interpreted** taking into account clinical information **and** laboratory data. " The reader will understand further **why** this test uses **whole** blood specimens in **preference** to serum, all the more **since** the aim of this test is to detect **transmissible** viral particles which should in **no** way be affected by the presence **or** the absence of blood cells.This is all the more **significant** since one of the **major** factors of HIV transmission is **supposed** to be Factor 8 administered to hemophiliacs and since this Factor 8 does **not** contain blood cells. Such a statement **implies** that it would be **impossible** to detect "viral" DNA in the **absence** of blood cells! ... in a test tube . **We are told, we can make "liters" of HIV and its DNA** (24), **but under surprising conditions.** It allows, among other things, the use of **plant extracts and other oxidizing chemicals, agents that could not exist in vivo.** The cell lines immortalized (**then patented**) by the teams of Mr Montagnier **and** Gallo are **co-cultivated with human cells or their extracts.** When the process is

finished, you **don't** get HIV itself; we simply demonstrate the presence**??** of an inverting transcriptase activity, which we take for **granted** to signal the existence of DNA which "must" be of viral origin. The **real** explanation for the phenomenon is as follows. In this mixture of cell cultures **and altered human cells (stressed),** there are **large** amounts of RNA and **inversion** transcriptase, because these cells have been **specially** selected and processed **to react in this way.** RNA is transcoded into DNA by the inversion transcriptase.
Long DNA fragments are then obtained which are **called** "**viral** DNA". They are actually fragments, **unrelated** to each other, of expressed RNA cells, transcoded into DNA and **linked to each other by** a process called "template switching" (one of the characteristics well known**??** in inversion transcriptase). (25) The **average** researcher **did not fail to believe that.**
It is **said** that this **linear** DNA is the **free or non-integrated form** of HIV, and **even** that **it is one of** the singularities of HIV, **because** such a quantity of free linear DNA has **never** been detected in **any other model.**

retrovirus. ... and by a **selection** process
At this stage, these DNA fragments are moreover shorter or longer than the "exact" **norm** of HIV. It is therefore necessary to **select** fragments of the "exact" size, otherwise this preparation **supposed** to be of specific DNA is **only** a magma of fragments of different sizes;

which transgresses one of the **fundamental** laws of virology, **namely** that all the nucleic acids of the **same virus** must be of identical dimensions.

... and by a detection process
These DNA fragments **artificially** selected to have identical dimensions are **not** yet in conformity. It is, at this stage of the operation, a **mixture** of all kinds of RNA fragments transcoded into DNA and which do **not** present a viral DNA of a unique specific model. It is then necessary to have recourse to a detection process (which evokes a combination lock), a **process which is called** "hybridization" (**hybridization**). Process in which DNA fragments are detected by a species of probe (probe) which collects them thanks to its **preselected** shape according to the shape of the piece of DNA that one **wishes** to find.

... by choosing a tailor-made probe
Since there is no DNA actually coming from HIV with which it would be possible to hybridize the DNA mixture, Mr Montagnier and Gallo **simply** use sequences (**stretches**) DNA which they **claimed** to be specific to VLTH-I (a retrovirus which Mr Gallo had previously **claimed** to have discovered) and which seemed to them suitable for this hybridization. The DNA thus detected was **duplicated**; certain stretches were cloned and **declared** to be DNA from VLTH-I (**later** called HIV). To

sum up: the aim of this exercise is the culture of HIV; but the **real** result is a mixture of fragments of different lengths of DNA (which goes **against** the virological law of identical dimensions), and is in **no** way a culture of virus. We are then **told** that the "**exact**" DNA was prepared by **isolating** certain fragments of this heterogeneous mixture by a hybridization process using a VLTH-I DNA probe , whose sequence is **known**. and **defined** as being similar to that of HIV. The fact remains that **in a truly correct** preparation, **no non**-hybridizing DNA fragment should be found. So the fact that we find these fragments, **proves** that it is a veritable potpourri of DNA fragments without any indication of the ..

It follows that the DNA of "HIV" **is only laboratory fabrication according to preconceived guidelines on what "must" be the DNA of a virus.** And **no one** seems to care that the virus itself **cannot** be reproduced, **whatever** the experimental conditions.

HIV DNA cloned by Montagnier and Gallo
It is not surprising that **no one has** long thought of spotting the **flaw** in the techniques used by the teams of Mr Montagnier and Gallo. After **assuming** that some DNA fragments are **specific** to HIV, each researcher worked with **only a few very** short cloned sequences (**never** on whole fragments), logically **assuming** that the **original** characteristics **had been properly reproduced.**

If we remember the process of identification and isolation that we have just described, it follows that in reality each of the sequences proposed for study **differs** from the others, which each researcher **did not fail to wrongly interpret it , as evidence of the legendary capacity for mutation on the part of HIV.**

A simulation of A little history.
Chapter 1 One of the **causes** of this lamentable state of things is perhaps due to the fact that VLTH-III was presented to the world, **during the famous press conference of** April 23, 1984 as being THE **cause** of AIDS (a commercial patent for a test the **same** day!). It is however customary in a true scientific procedure to submit to the examination of his peers , **evidence** of what is said before any public disclosure. This unhealthy haste can be explained by historical circumstances: a disagreement between the National Cancer Institute **and** the Center for Diseases Control (CDC) which was at the time in **favor** of the French thesis on the virus. The CDC had, by chance, **made its position known the day before the press conference,** in a large front page article in the New York Times: CDC management said that the virus identified by the French was the **cause** of AIDS. (27)
Chapter II Even under these conditions, one cannot help admiring the **audacity** of Mr. Gallo: in **1975**, he **claimed** to have discovered the first human retrovirus (LH23) using the **same** techniques. It was **later** discovered that

this LH23 was **nothing** more than an assembly of **three** different DNAs from **three** different sources of contamination. (28) Nowadays, even a first-year student knows that by incorporating 'DNA in a cell culture, part of this DNA is incorporated into cells **without** any virus being involved.

What does the AIDS test actually detect?
Since "HIV" **is a laboratory artifact,** we must **infer** that, when it is **not** just a reaction with **other antibodies**, the "AIDS" test detects antibodies to proteins **produced by the test process itself.** These proteins must be **of human origin** because the cells originally used **come from** people with leukemia. Immunological contact with these cells logically leads to a **positive** test. Since, however, a **positive** reaction actually covers **other** unrelated factors, such as **rheumatism** or **sunbathing**, **no** specificity can be attributed to this test. (29) Furthermore, the commonly accepted relationship between a **positive** reaction to antibodies and a given disease **still needs to be confirmed by a critical re-evaluation of the data on this subject.**

Therefore, condoms can only protect against venereal disease (or serve as contraceptives). Even worse, they lull the user into a false sense of security by ignoring the real dangers to which they may be exposed.

AIDS research must change focus

AIDS research is therefore at the start and not at the stage of fundamental discoveries as we have suggested. (30) Since 1993, the great tenors of the start have been sidelined by **skillfully** suggesting that the virus has **mutated** so much that **it is no longer** detectable. AIDS **must therefore be explained "in the absence of a whole virus available".** (31) In addition to the **aberrations** of the tests, **other** errors such as the counting of **T** cells contribute to **worsen** the situation. In fact, **the whole concept of AIDS needs to be rethought, redefined.** (32) We must, for example, recognize that it is completely **useless** to rename AIDS a whole collection of ancient and well-known diseases, under the **pretext** that the patient is positive for the antibody **or** genetic test (PCR). By eliminating the **hypothesis** of the existence of an HIV virus, we understand why the epidemiological projections which promise, for years, a pandemic, have been so belied by the facts. In 1986, Africa was presented as a terrible warning: that was what awaited the Western world. In Africa, AIDS is diagnosed **without** an HIV test by the mere presence of a combination of clinical **signs** (33), chronic fevers, cough, diarrhea and weight loss. All of these symptoms are **just** those of the most common disease: **poverty**. (34) It would certainly **not** be surprising if a totally **different** definition produced a **different** result.

Finally, the effect that the announcement of a positive test has on the **mental** and physical health of a patient must be taken into account and evaluated in order to no longer unnecessarily **frighten** people. Misinformed people not knowing what HIV and AIDS are. (35)

Anti-viral drugs

In addition, stop using AZT and the other "anti-virals" that are supposed to stop the spread of HIV. Indeed, these substances indiscriminately destroy all cells and ultimately **destroy the whole body.** Note an alarming fact: AZT and other similar products preferentially attack rapidly dividing cells, that is to say intestinal cells (causing diarrhea and **poor** absorption of food) and (sad irony) the cells of the **spinal** cord, the main production site of cells of the immune system. (36)

The large number of HIV positive people, whose condition **cannot** be explained by the official theory of AIDS, just as much as the phenomenon of sero-conversion (return to seronegativity) is eloquent proof. Researchers and medical authorities have a duty to openly and humanely debate the "HIV / AIDS" relationship and to recognize that it is a **mistake** to think that an immune deficiency can be **caused** by an infectious agent.

The future

In order to live a fully fulfilled life, we must first find and then maintain an autonomous management of our body and our health, by snatching them from the hands of the **self-proclaimed experts** who have dispossessed us of them. (37) If we **refuse** to learn from the case of AIDS and the drifts in research and health policies that it has caused, the **worst is still to come, and in the too soon future.** (38) The genetic program set in motion in the 1860s (39) and a primary genetic **determinism** are already accomplished. The **so-called** availability of genetic sequences and the pretensions to manipulate them easily make some scientists lose their heads, who, blinded by their **ambitions**, **mold** the truth as they wish (40). All genetic **models** and the technologies that flow from them, such as **gene therapies, are based on** one-dimensional static models that constitute a gross, primary and outrageous **simplification**.

You have to remember that the father of modern genetics, Gregor Mendel,

Acknowledgments : this article is dedicated to Ivan Illich and Thomas McKeown: **if their writings had been taken more seriously, the great panic of AIDS and many other perversions would have been spared the world.** I also want to thank Volker Gildemeister (Meditel, London) for his translation and constructive criticism and of course my family, Hans-Walter Wiegand and many other friends,

too numerous to mention, for their collective support. Stefan Lanka 1995. Translation: Françoise Louis for Mark Griffiths. References 1 Klemens B. Meyer and Stephen G. Pauker. 1987. Screening for HIV: Can we afford the false positive rate? NEJM 317: 238-241. See also: Marsha F. Goldsmith. 1985. HTLV-III testing of donor blood imminent; complex issues remain. JAMA 253: 81-86, 173-175, 179-181. 2 John Crewdson. The Great AIDS Quest. Special report. Nov. 19, 1989. Chicago Tribune. 3 Frankel, Mark; Mary Hager, Theodore Stanger. July 25 1994. The End of a Scientific Feud. Newsweek. 4 Eleni Papadopulos-Eleopulos, Valendar F. Turner, John M. Papadimitriou. 1993. Is a positive Western Blot proof of HIV infection? Bio / Technology I 1: 696-707. 5 A similar article was published in a German monthly: Stefan Lanka. 1994. Fehldiagnose AIDS? Wechselwirkung, Aachen, December, 48-53. 6 Temin HM and Mizutani. 1970. Viral RNA-dependent DNA polymerase. Nature 226: 1211-1213. Temin HM and Baltimore D. 1972. RNA-directed DNA synthesis and RNA tumor viruses. Adv Vir Res 17: 129-186. 7 Gerald B. Dermer. 1994. The Immortal Cell: Why Cancer Research Fails. Avery Publishing Group, Garden City Park, NY. Gerald B. Dermer. 1994. Another Anniversary for the war on Cancer. Bio / Technology 12: 320. 8 Gye WE and WJ Purdy. 1931. The cause of Cancer. Cassell, London. 9 Weiss R. et al. 1982. RNA Tumor Viruses. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York. 10

Bishop JM 1978. Retroviruses. Ann. Rev. Biochem. 47: 35-88. Bishop JM 1983. Cellular oncogenes and retroviruses. Ann. Rev. Biochem. 52: 301-354. Doolittle RF et al. 1989. Origins and evolutionary relationships of retroviruses. The Quarterly Review of Biology 64: 1-30. Varmus H. and Brown P. 1989. Retroviruses. In: Mobile DNA: 53-108, eds .: Berg E. and Howe MM American Society for Microbiology. Washington DC Coffin JM 1990. Retroviridae and their replication. In: Virology. Fields BN ed., New York. Doolittle DF et al. 1990. Retrovirus Phylogeny and Evolution. Current Topics in Microbiology and Immunology 157: 1-18. 11 Why we will never win the war on AIDS. Ellison BJ & Duesberg PH 1994. Inside Story Communications, E1 Cerrito CA. 12 John Higginson, Calum S. Muir, Nubla Munoz. Human Cancer: epidemiology and environmental causes. Cambridge University Press. Samuel S. Epstein. 1992. Profiting from Cancer. Vestod Interests and the Cancer Epidemic. The Ecologist 22: 233-240. Samuel S. Epstein. 1993. Evaluation of the National Cancer Program and proposed reforms. International J. Health Services 23: 15-44. 13 Tim Beardsley. 1/1994. A war not won. Scientific American 70: 118. 14 see ref 11 15 Malcolm A. Martin et al. 1981. Identification and cloning of endogenous retroviral sequences present in human DNA. PNAS 78: 4892-4896. Tl Bonner et al. 1982. Cloned endogenous retroviral sequences from human DNA PNAS 79: 4709-4713. Callahan R. et al. 1982. Detection and cloning of

human DNA sequences related to the mouse mammary tumor virus genome. PNAS 79: 5503-5507. Temin HM 1985. Review: Reverse Transcription in the Eukaryotic Genome: Retroviruses, Pararetroviruses, Retrotransposons, and Retrotranscripts. Mol. Biol. Evol. 2: 455-468. Harold Varmus. 9/1993. Reverse Transcription. Scientific American 257: 48 16 Dixie L. Mager and Paula S. Henthorn. 1984. Identification of a retrovirus-like repetitive element in human DNA. PNAS 81: 7510-7514. Catherine O'Connell et al. 1984. ERV3, a full-length human endogenous provirus: chromosomal localization and evolutionary relationships. Virology 138: 225-235. Baltimore D. 1985. Retroviruses and Retrotransposons: The Role of Reverse Transcription in Shaping the Eukaryotic Genome. Cell 40: 481482. Paulson KE et al. 1985. A transposon-like element in human DNA. Nature 316: 359-361. Callahan R. et al. 1985. A new class of endogenous human retroviral genomes. Science 228: 1208-1211. Weiner AM et al. 1986. Nonviral retrotransposons: Genes, pseudogenes, and transposable elements generated by the reverse flow of genetic information. Ann. Rev. Biochem. 55: 631-61. Dixie L. Mager and Douglas Freeman. 1987. Human endogenous retrovirus-like genome with Type C pol sequences and gag sequences related to human T-Cell Lymphotropic viruses. J Virol. 61: 4060-4066. Shih A. et al. 1989. Detection of multiple, novel reverse transcriptase coding sequences in human nucleic acids:

relation to primate retroviruses. J Virol. 63: 64-75. Krause H. et al. 1989. Molecular Cloning of a Type D Retrovirus from Human Cells (PMFV) and its Homology to Simian Acquired Immunodeficiency Type D Retroviruses. Virology 173: 214-222. Wilkinson DA et al. 1990. Autonomous expression of RTVL-H endogenous retrovirus-like elements in human cells. J Virol. 64: 21572167. Banki K. et al. 1992. Human T-cell lymphotropic virus (HTLV) -related endogenous sequence, HRES-I, encoded at 28-kDa protein: A possible autoantigen for HTLV-I gag-reactive autoantibodies. PNAS 89: 19391943. Horwitz MS et al. 1992. Novel Human E
www.sidasante.com/science/scistef1.htm

Barré-Sinoussi was named an **Officer** of the National Order of the Legion of Honour in 2006 and was raised to **Commander** in 2009. She was promoted to the dignity of **Grand Officer** in 2013.

Rethinking HIV. About how "HIV" is a **byproduct** from the world of retrovirologists. Therefore, the burial of "HIV" **will mean the end of all retrovirology.**
Dr Stefan Lanka.

Here is a terrible example of how a distinguished academician who has contributed much to the

advancement of science and **who then prevents further advancement by his obstinate adherence to a dogma of his own creation.** If he hadn't felt compelled to repeat incredible things just because they were said one day, he would have happened to be a completely different person. Joan Wolfgang von **Goethe**, maximen und Reflexionem. Textelle 586.

Readers should be aware that there have been a number of reactions, and corresponding responses, to the bonus offered by the English magazine Continuum as a reward for the "**Missing** Virus". This goes from clarification petitions, such as what type of evidence is required, to ironic comments about the irreverence of the evidence requested , to a request across the price line from Peter Duesberg. Readers will remember that the starting point for this whole **movement** was **my article** explaining that in reality "HIV" does **not** exist, presented as opposed to the most frequently asked question, whether or not "HIV" is not responsible for AIDS. The Australian scientific team led by Dr Eleni- Papadopoulos has **already** developed a detailed response to Dr Duesberg's request, for which I would devote myself to addressing **how the mistaken concept of "retrovirus" is** decisively influencing the present situation. The enormous service Peter Duesberg has rendered to humanity is beyond discussion. since 1987, it has been largely at its own expense and firmly, the bastion of wisdom and decency in

a world brought to madness by the simplistic theory of **HIV = AIDS.** Whether "HIV" exists and is the cause of "AIDS" are academic questions: "when was the last time you met a" normal "heterosexual - that is, someone whose life does not depend on the perception of the panic surrounding "AIDS" - who pays the least attention to the official history on "AIDS"? In practice, Dr. Duesberg's merit for our gratitude is his convincing and unwavering opposition to AZT (and other similar products), the use of which is **deadly**. That said, it is **also certain** that even Dr Duesberg is the **victim** of another collective deception ("the denkkolectiv", **collective thought elaborated by Ludwig Fleck**) which he **himself** helped to formulate, and in which he apparently finds himself **prisoner**.
The **retroviruses were postulated as** a species of microorganisms that cause reverse transcriptase, which was **entirely** reasonable in the early **1970's** as a working **hypothesis**. The **error consisted in raising the hypothesis to dogma.** The **first** techniques of genetic detection gave a certain credibility to the existence of an entity which would be transmitted from one cell to another, which was unlucky, **because it turned out later that this was wrong.** Errors of this type always occur when technology puts a new experimental process within the reach of general use which pushes a battalion of researchers to the massive production of experimental data, **neglecting** the biological significance that their work can have, **if** there is one. Even **worse** is the habit of

making an **endless** number of **ad hoc** readjustments of the **original** theory, which **completely** distorts this one. **Rigorous** science demands that the problem be fully rested when it happens. **If** this is not done, as is the case with "AIDS", we continue to advance in the greatest confusion on fundamentally **wrong** bases, and it is a **disaster**. Dr. Duesberg has been in the main research on "AIDS", **limiting** his objectives to the relative **minor** aspect that "HIV" may or may not cause "AIDS", **when what should really have been the audacity to question the very concept of retrovirus,** given his previous and **courageous** position well before any other, and to **admit** the **error** of the **hypothesis** of **retroviruses** as a cause of **cancer**, **despite** having been involved for many time in this other deception. From my point of view, it is reasonable to consider that Dr. Duesberg could come to convince himself that there is **no entity such as a retrovirus.** But instead, he let himself be seduced by the technical prowess of "**retrovirologists**", capable of reproducing consistently certain particular phenomena of determined biological constituents of cells. By doing this, he allowed himself to be **misled** into the belief that the said phenomena were due to a virus. It is a **complete non** "sequitur". According to a modern metaphor, this **lack of intellectual rigor** has transformed **molecular** biology into a virtual science **and** presented "HIV" as **due to a virtual pathogen.** **Un**fortunately for humanity, "AIDS" is **not** the only aspect, except that it represents

only the tip of the iceberg. is left **misled** in the belief that the said phenomena were due to a virus. For a discerning observer it could have been clear **already in 1973** that it was **im**possible to support the working **hypothesis** which attributed **to** retroviruses the observed experimental phenomenon of **reverse** transcriptase, when it appeared that said reverse transcriptase was anything **except** a **limited** phenomenon. At most in 1980, this hypothesis should have been **abandoned** by everyone. In fact, the extraordinarily **artificial** and circumstantial conditions that can **induce** reverse transcriptase in **laboratories** should have **warned** anyone about the **extreme improbability** that such **exclusively** laboratory conditions have any meaning for phenomena that occur naturally. . Even more when it was impossible to show the existence of **no** retrovirus, for example by being able to **isolate** and characterize it, **and** to demonstrate its transmissibility. These failures, (obviously **not** for lack of attempts) should have been sufficient to **abandon** all this focus. It can be difficult to admit that all the cards which **claim** to represent a complete retrovirus, included for "HIV", are **only** compilations of pieces and pieces put together by their authors **to the delight of their beliefs.**

Among colleagues ... **neither in vivo nor in vitro,** it has **not** been proven that there is **no** retrovirus, or its RNA in its entirety! An **additional** difficulty for the **HIV = AIDS hypothesis** is that it has **never** been possible to prove that

the experimental observations attributed to retroviruses are **exogenous** to the cells used in their experiments, that is to say, which come from outside. of the cell. In reality, all the available evidence attests to the contrary, that is, that they are endogenous (**inherent**, internal) to one's own cells. **Part** of the evidence is that the **so-called** retroviral activity could **only** be induced experimentally in **one specific type of cell,** while it is **assumed** that "HIV" infects **many** distinct types of cells in the body. The two statements are clearly **incompatible**. The whole theory becomes even **less** plausible when one keeps in mind that the "retroviral" concentrations are always **extremely low** and that a **large** quantity of patients' cellular material is **necessary** to be able to have proof that there is a "replicating virus. ". By the way, this is the **basis** of the **claim** that "HIV" has a **very low infection rate** ... **A more rational explanation is that there are no viruses.** History unfortunately offers a **precedent** in this form of research. At the end of the 19th century and the beginning of the 20th century, **a long series** of experiments with **highly** (endogamized?) Laboratory animals. Under **strictly** circumscribed conditions, they developed a greater **susceptibility** to the disease than **non** (endogamous) animals. The phrase "highly endogamized" was forgotten/**omitted** and a **generalization** was **declared** on viral infectability which proved to be **wrong**, but which medicine remains trapped today. Analogously, **experiments are carried out today with cell cultures**

instead of doing them with complete animals, and that for the simple reason that in this way one can enormously accelerate these experiments. The **disadvantage** is that it **limits** the experience to only one of the few cell lines that are **always** cancerous because they only grow in the **laboratory**. **History repeats itself:** the results obtained with highly **ab**normal cells are **generalized** according to the behavior of normal cells. These cells can incorporate into their own DNA bits, of foreign DNA that mix with growth **cultures** (an integration process that normal cells can **also** carry out, albeit more **slowly**). The cells which have incorporated the DNA, will manifest, as it is evident, the characteristics which codify the said DNA, which is **interpreted** as that a virus went into action **when nothing** like this happened. From there, it is **easy** to realize the **strange** appearance of the **concept** of "infectious DNA", and the **erroneous conclusion** that in the process is involved a **virus**, according to the conventional sense of meaning of this word. No doubt, all the argument aborts when we demonstrate that , we can make that **non**-viral DNA can do so, **both in vivo and in vitro.** **If** in this case the DNA used is DNA which has **arbitrarily** been defined as DNA of "HIV" or a part of it, This is the basis of Dr. Duesberg's claim. In his retrovirologist **jealousy**, he does not seem to realize that "infectious DNA" **is a contradiction in his own terms.** Why ? **What is normally called a virus, if not** a piece of DNA wrapped in a protein blanket, so that DNA can be transmitted from

one cell to another? A piece of DNA filament can**not** do this by itself, because it would be **exposed** to enzymatic degradation **or it** would be mixed with other components. Besides, **how** could he identify his Dna cell? **How** could he reach it? **How** could he enter it without a mechanism that allows it?

CONCLUSION: The rules that demonstrate the existence of "HIV" (and retroviruses in general) have **never** been followed by the very people who invented them, as they have **never** been validated. This now makes it easier to understand why many people feel the need to ask what the term "isolated" **means** in sufficiently obvious terms: adequate synonyms could be "pure" and / or "free of contaminants". A concern clearly arises in their minds when they realize that the **term** isolation has been used in retrovirology in the way stated by **Alice** in Wonderland: "it means what I say it means".

Until the invention of "AIDS", retrovirologists were a small minority sect and were happy to accept the **fantasies** of each of them without criticism. They could continue playing the violin to the delight of their hearts, quietly, knowing that "retroviruses are the least dangerous of all viruses". Well-meaning and gullible colleagues, such as **so-called** virologists, journalists and through them, the general public, were **hypnoptized** by the **incomprehensible** jargon of **retrovirologists**, in the

belief that the immense mass of data accumulated on "HIV" **and** retroviruses mean anything. In **reality**, it can be shown that **each** property attributed to "HIV", **and** to retroviruses in general, **belongs** to cells used in co-culture experiments. At **no** time has there been any **solid** basis to believe that these properties and components have nothing to do with viruses in general or with "HIV" in particular. **No** "HIV" particle has ever been obtained pure, free of contaminants. It has **never** been proven the existence of a complete part of the RNA attributed to "HIV" (nor of the transcribed DNA).
Extract from Continuum, vol 4, n 3, Sept-Oct 96.
Translation Sylvie Cousseau, 1999.
www.sidasante.com/science/scirevih.htm

PHOTOS OF HIV What they actually show. By **Stefan Lanka** (virologist, Dortmund)
Translation Dr Marc Deru
It has long been known that what "AIDS" researchers have presented as pictures of HIV is actually normal cellular particles responsible for metabolic transfers or other functions. As these particles have, unlike viruses, only intracellular functions, they are very unstable once extracted from their cellular environment, and they cannot be isolated or photographed in an isolated state. ((True viruses are so stable that it is easy, to prove that they have been isolated, to photograph them directly, in 3 dimensions, with an electron microscope (ME) without

having to resort to **prior chemical** fixation-theoretically)) On the other hand, the particles charged with metabolic transfers and the other cellular particles are so unstable that they can only be photographed **after chemical** fixation, in cells, tissues or special culture media. (The exception to this rule is mitochondria, cellular organelles producing energy, which can be isolated in a stable form). These particles cannot therefore be **isolated**, they are accompanied by other elements of cells, tissues or liquids **chemically** fixed and embedded in the resin (resin-embedded); to visualize something at EM, you have to make ultra-thin cuts in this mixed material, because at EM you **can't** see anything if the cuts are thicker. Of course, existing viruses can also be photographed in ultrafine sections, but this is the important point - in an isolated state . **All that has been presented to us as HIV is ultrafine sections of cell particles.** (1, 2) Fig 1 ME photo, in ultra-thin section, of very small particles. These particles are **claimed** to be HIV, but in reality they are cellular, **not** viral, particles (**usually called** "virus-like particles", "microvesicles", "microsomes"). The debris at the bottom of the photo shows that the particles are **not** purified or isolated. **These photos are always published without any evidence that the particles are of viral origin.** Fig 2. EM photo, **enhanced** by computer, of a cell surrounded by small particles. The blue / gray colored particles that are **claimed** to be HIV particles attacking or leaving (depending on publications) a white blood cell are

in fact **artefacts** due to the staining and / or fixing process; **at best** it can be cellular particles entering or leaving the cell (phenomenon well known as endo or exocytosis). Beautiful photos like this (made by Lennart Nilsson) have been published **without any evidence that the particles are of viral origin.** Fig 3 HIV researchers **believe** that the "AIDS" virus **looks like this: a bomb or an underwater mine in the blood.** This model is based on the detection of cell particles in cell lines **cultivated under very special conditions.** Such particles have **never** been isolated, and their **existence as a virus or** particle of viral origin has **never** been demonstrated in any way. This is just a **model** based on a set of proteins of various sizes (no other characteristics!) Which are, by **anti**-body tests (known as HIV tests or AIDS tests),**supposed** to represent parts of HIV. This **model** was **built on a misconception of what** a retrovirus is **and what it should** look like.
References : 1. Stefan Lanka: Fehldiagnose AIDS? Bisher konnte das AIDS-Virus nicht isoliert werden. Wechselwirkung , 48-53, Dezember 1994. Stefan Lanka: HIV - reality or artefact? Continuum Vol 3, No 1, 4-9, April / May 1995. Stefan Lanka: HIV debate. Continuum Vol 3, No 2, 4-7 + 27-30, June / July 1995 2. Eleni Papadopulos-Eleopulos, Valendar F. Turner, John M. Papadimitriou, David Causer: The isolation of HIV: Has it really been achieved? The case against. Continuum Vol 4, No 3, Supplement 1-24, September / October 1996 www.sidasante.com/science/scihivph.htm

"HIV" HAS NEVER BEEN ISOLATED Namur - October 12, 2002. Etienne de Harven

In AIDS dissent circles, you will often hear the claim that HIV has **never** been isolated. This claim is categorically rejected by well-meaning orthodoxy as belonging to the realm of heresy. It is often debated in a somewhat laborious manner by dissidents, the difficulty being due to the fact that the word "isolation" **is not always given the same definition.** To better understand this debate, we will spend a few moments reviewing together **what is meant,** in classical virology, by the words "isolation" and "purification". On the basis of which, we will conclude on the application of these terms in the specific case of HIV.

In experimental pathology, working on well-selected chickens or mice, several diseases including various forms of cancer and leukemia can be **transmitted by injecting** these animals with "acellular filtrates". These filtrates being **totally** devoid of cells and bacteria, such experiments made it possible to make a clear distinction between the transmission of cancer by cell transplant, or by infra-microscopic factors such as viruses. Such filtrates were obtained by various ultrafiltration techniques, techniques which completely eliminated the presence of whole cells or bacteria. If, moreover, the activity of such filtrates were concentrated by centrifugation at high speed, maintained after storage at low temperature, but lost by heating at 65-68 °, it could reasonably be

concluded that the disease in question had been transmitted by a virus. And it could be said that the virus in question had been "isolated". It was such a methodological approach that allowed **Peyton Rous** to "isolate" the chicken sarcoma virus, and **John Bittner** to "isolate" the virus from mouse mammary tumors, all **before** the invention. and the application of electron microscopy to experimental virology. When similar experiments are carried out, **no longer on laboratory animals but on cell cultures,** it is possible to demonstrate the "**isolation**" of a virus, **a result which is then based on** the observation under the microscope of various cellular **alterations**, such as formation of giant pluri-nucleated cells. **And it was experiments of this type that enabled** Luc Montagnier's research group, at the Institut Pasteur in 1983, to **isolate** a retrovirus initially called LAV and **renamed** "HIV" soon after. The **difficulty** of **interpretation**, in the case of the discovery of the Montagnier group, stemmed **from** the fact that the cell **cultures** used were very **complex**, **in fact comprising a mixture of several cell types, some of which are well known as being chronic carriers of retrovirus.** There has indeed been an "**isolation**" of a retrovirus, that is. But there was **no** evidence that this "isolation" had **any** relation to the infection of cell cultures with extracts from an AIDS **patient**. In short, there was **probably** "isolation" of a retrovirus, but there was **no** reason to **claim** that this virus came **from** the

patient, and therefore **no** reason to call it HIV, which brings us back to the title of my presentation: HIV has **never** been isolated! But there is **another** problem related to the **interpretation** of this type of viral isolation. The problem is that **this** type of isolation does **not** in any way identify with **certainty** molecules that could be considered as molecular "markers" **specific** to the virus. Because, in fact, **to** identify with **certainty** molecules that could be considered as **specific** "markers" of a virus, this virus must **first** be highly purified, that is to say separated from any contamination by cellular or bacterial debris. The success of such purification must be rigorously tested, failing which the identification of so-called "markers" is serious scientific **fraud**. It is here that electron microscopy takes an essential**????** role, because to test the success of a purification technique, and despite all the noise made around the techniques of **molecular** biology, it **remains** the **only** method which makes it possible**???** to demonstrate that the virus particles have been successfully separated from any cell, bacterial or mycoplasmatic contamination. **Two** methods have been used **successfully??** to **purify** viruses. **One** is based on **ultrafiltration**, the **other** on **high speed centrifugation** in density gradients. In my research on the mouse leukemia viruses (**Friend** leukemia), I used a combination of ultrafiltration and centrifugation methods which allowed us to demonstrate, in 1965, a remarkable degree of purification of the Friend virus (1). I have **never** used

the **gradient** techniques, used however **with great success** by other authors whose work had clearly demonstrated that the carcinogenic RNA viruses (**as they were called before 1970**) all sediment, in sucrose, at the density of 1.16 gm / ml. The **problem** with the gradient method was clear, however: it was well recognized that many cellular debris, such as microvesicles, **also** sediment at this **magic** density of 1.16gm / ml. Harvesting material at this density is therefore **not** enough to proclaim the isolation of a retrovirus, its far from it! The need to control the **absence** of cellular debris by electron microscopy**????** is therefore an **absolute** necessity, a fact which was clearly **reaffirmed** in 1973, **at** the Institut Pasteur, during an important conference which dealt **exclusively** with methods of purification of retroviruses (2).

Before considering the implication of these remarks in the **alleged** "isolation" of HIV, we must return to a **major event** which took place in 1970, that is to say the discovery by Temin (3) and by Baltimore (4) reverse transcriptase (that is to say DNA synthesis **from** an RNA model). It was a revolution in molecular biology to understand the activity of this **enzyme** which we very **aptly???** called reverse transcriptase (RT). In 1970, the transcription of RNA into DNA was certainly a surprising discovery. A discovery which provided a very attractive explanation for the possible mechanism of action of

carcinogenic RNA viruses! Reverse transcription had **never** been observed in biology before 1970, and the interest in this discovery was such that it was decided, soon after, **to re-baptize** carcinogenic RNA viruses under the **name** of retroviruses… But where was the **problem.** The problem was that Temin **and** Baltimore had **neither** verified the **purity** of the virus samples **in which** they identified the **enzyme** activity in question. However, shortly after their publication in 1970, it became **evident** that reverse transcription was a **very common** phenomenon in biology, as summarized by **Varmus** in 1987 (5). As early as 1971 (6), it appeared that reverse transcription was common to a large number of animal cells, as **well** as bacteria (7). Therefore, **before** considering the enzyme as a retroviral marker, it would have been necessary to repeat the experiments of Temin and Baltimore on samples whose degree of purification would have been **verified**, in order to exclude the presence of cellular **debris** which could , by themselves, **explain** the presence of reverse transcriptase activity. To my knowledge, these controls have **never** been carried out, and the enzyme has been considered for **30 years as the main marker** of retroviruses! In the **historical** article published by Barré-Sinoussi, Chermann, Montagnier and collaborators (8) and in which the **isolation** of a retrovirus was **announced**, the detection of enzymatic activity (RT) in a fraction sedimenting at 1.16gm / ml was the **key** to demonstrating a retrovirus. **Now we know** that this

enzyme is **not** a specific marker for retroviruses! And we have known for a long time that the 1.16gm / ml fractions contain an **abundance** of cellular debris **perfectly** capable of explaining the presence of enzymatic activity.

Barré-Sinoussi's article also made much of an electron microscopy image illustrating retroviruses budding on the surface of a lymphocyte. The image was **interpreted** as evidence of infection of the **cultured** cells with the extract from the **patient**. What the article **failed** to consider is that the cultures were **mixed** with lymphocytes from the umbilical cord blood, and that the human **placenta** had been known for several years (9) as a tissue exceptionally rich in endogenous retroviruses (HERVs).

In short, the article , considered **worldwide** as the **basic** reference on HIV isolation , is **based on three methodological errors:** 1) Not having verified the presence of cellular debris in the fractions, 2) having ignored the enzymatic activity **of** these same cellular debris, and 3) having ignored the presence of endogenous retroviruses in the cells in culture.

This article can be**??** considered as the demonstration of a retrovirus, probably endogenous to the cell cultures used. However, it **cannot** be presented as evidence of the isolation of a retrovirus from an AIDS patient.

It took 15 years for the **first** experimental controls to be carried out in **two** laboratories, one in the United States (10), the other in France (11). These two laboratories published jointly, in Virology, their results of study , under the electron microscope , of gradients obtained **from** cell cultures **supposed** to produce HIV. In both cases, the authors observed an **abundance** of cellular debris, with **no acceptable evidence** of retroviral particles. At around the **same** time, Luc **Montagnier** was interviewed by Djamel Tahi and finally admits that, in fact, HIV had **never** been purified in his laboratory… (12). It is interesting to note that in the article from Pasteur in 1973, it was **clearly** indicated that reverse transcriptase activity is **present** in cell debris. As incredible as it may seem, it is in this **same** laboratory of the Institut Pasteur that, ten years **later**, in 1983, the role of cellular debris was **ignored**, putting AIDS research on a false track for them. 20 years to come… Before concluding, I would like to add a few remarks relating to **other** molecular "markers", **also** relating to the **alleged** genomic isolations **based on** PCR techniques, and finally relating to the **abuse** of public credibility by images, embellished by **computers**, which are said to represent HIV.

Other molecular markers. Let us not return to the reverse transcriptase which has already caught our attention. Several proteins, **allegedly** of retroviral origin, are

frequently used as **specific** "markers", for example p24. The doubt, already old, on the **specificity** of this marker was clearly analyzed recently by **Fabio Franchi** (13) who underlines the **absence** of any correspondence between the results obtained with p24, **and** the measurements of the **alleged** "viral load" **supposed** to be measured by PCR. On the other hand, one **cannot** fail to be disturbed by learning that 50% of **dogs**, tested by the Western blot, react positively with one or more of the HIV proteins obtained by genetic **recombination**, such as gp120, gp41, p31, and p24 (14).

The **lack of specificity** of these **so-called** structural proteins of HIV was clearly demonstrated, almost 10 years ago, by Eleni Papadopulos and the group from Perth in a classic article published in 1993 in Nature / Bio-technology (15). The authors' conclusions could not have been clearer, but have been cautiously **ignored** by the AIDS orthodoxy. A constituent of normal cells such as **actin** probably corresponds to gp41, while gp120-160 probably corresponds to an oligomer of gp41. Obviously, the presence of cellular **debris** easily explains the presence of **so-called** retroviral "markers". And **all of the so-called HIV isolation successes are easily explained by the misuse of non-specific markers.** As we have already pointed out, Gene markers **and** viral load measurement by PCR . This approach could, **in principle**, **appear** more attractive for two reasons: 1) it applies directly to the **blood** of patients, thus avoiding all

the uncertainties that surround the **cell cultures,** and 2) the methods are **allegedly** qua**nti**tative.

Note that it has **never** been possible to visualize the smallest retroviral particle in the blood of AIDS patients under the electron microscope, **even** if patients with **very high viral load** are selected (16). In addition, it seems **likely** that PCR techniques are capable of amplifying small RNA fragments **from** gene fragments of endogenous retroviruses, which would be expressed more abundantly under **stress** conditions. It should be noted that **more than 2%** of the human genome is represented by endogenous retroviral sequences**????** (17, 18).

Measuring the **so-called "viral load" by** PCR may well have **no** connection **with** the quantitative measurement of a **so-called** exogenous HIV. Finally, Let us not forget the **absence** of any correlation between the **so-called** measures of **viral load** and those of the number of p24 molecules in the circulating blood of patients. **Nor** should we forget that **Karry Mullis** himself, the discoverer of the PCR technique who received the Nobel Prize in 1993 for this, categorically **rejects any application of** "his" PCR technique **to quantitative measurements of** HIV ... (19).

The abuse of beautiful images. You can find in newspapers and magazines around the world **admirable** images, colorful and totally **artificial**, which are

supposed to represent HIV itself, embellished by **computers**. To publish such images is to bring to the attention of the general public and doctors an apparently limpid and clear message**:** HIV **has indeed been well isolated since it can be seen and portrayed under the electron microscope! This is a huge lie!** All of these images come **from** cell cultures. **None** comes directly from a single AIDS **patient** (20), **even if** one applies to selecting patients labeled as having a high viral load. Luc **Montagnier** himself described the very complex cell **cultures** used for HIV **as real soups of retroviruses** (12)! And it's true! This is true, because everything had been **planned** for retroviral particles to appear there, and because the **basic** checks which **should** have made us understand the **real origin** of these viruses were **never** done, or if they were done have **never** been published! These cell cultures are **always mixed and hyper stimulated.** Mixed, because they consist of a **clever mixture of several cell lines including,** for example, the patient's lymphocytes plus H9 Gallo cells, cells which are well known as **chronic** carriers of retroviruses (21), or lymphocytes of the patient **mixed** with umbilical cord lymphocytes which, derived **from** the placenta, are likely to carry **endo**genous retroviruses. An example of **excessive** use of the convincing force of a beautiful image is provided by the classic article by Barré-Sinoussi, Pasteur 1983. We see an excellent image taken there under the electron microscope and representing particles

of retrovirus budding on the surface. of a lymphocyte. Perfect ! But the authors use this image to prove that this lymphocyte was infected with the patient's viruses. Gold, **nothing proves this interpretation!**
Everything suggests, on the **contrary**, that the endogenous retroviruses of this lymphocyte from the blood of the umbilical cord were **activated by the specific conditions of the culture.** All these **mixed** cultures are **also hyper stimulated by various growth factors such as phyto**hemagglutinin (PHA), T lymphocyte **growth factor** (TCGF), plus **Interleukin**-2, or even more **corticosteroids**. However, all these factors are known to **activate** the expression of endogenous retroviruses that we all carry**???** within us (18). Should we therefore be surprised to observe retroviral particles **in such hyper stimulated "retroviral soups"?** No, certainly not. It is very disturbing to note that in this historical article of 1983, electron microscopy **was not used when it was essential**??? to identify cellular debris, and was **misinterpreted** in the case of umbilical cord lymphocytes.

Conclusion.
In fact, HIV has **never** been isolated or purified. Retroviral particles, **most likely** of endogenous origin, have been observed in cell **cultures**, but their **hypothetical** link with AIDS **patients** has **never been proven,** little more than their pathogenicity. For **political**

and unscientific reasons, reasons for which we explained ourselves this morning, the AIDS orthodoxy tried to face this difficulty of isolating the virus directly by **inventing** several molecular "markers". Because the HIV = AIDS **hypothesis had to be saved at all costs** (22), **even at the cost of scientific integrity** (23)! We have seen, however, that these markers, which totally **lack specificity, have not led to any consistent observation.** **If** AIDS was indeed a disease caused by a retrovirus, how is it that 20 years of research have **not** made it possible to isolate the **exo**genous retrovirus responsible? How is it that what was so **easily** demonstrated in mice is so **difficult** to demonstrate in humans? **Twenty years of effort based on a single hypothesis,** the HIV = AIDS hypothesis. Twenty years of effort to reach, in 2002, no curative treatment, no vaccine, and **no verifiable** epidemiological prediction… Do you not think it is high time to **courageously** ask the essential question? The question being: is the HIV = AIDS hypothesis correct? Because there is a way to see AIDS differently, **outside** the limited framework of infectious diseases and retrovirology. Let's think about toxicology, pharmacology, **malnutrition**, **stress**… And from this perspective, which is full of optimism, the difficulties encountered in the efforts to isolate and purify the so-called HIV find a simple and totally disarming explanation: these difficulties probably result **from** the fact that **HIV DOES NOT EXIST** as an exogenous and infectious agent. Doubt about the very

existence of HIV has been raised by several scientists for several years (24, 25). But, for orthodoxy, we had to remain politically correct, even if we had to invent HIV to try to justify large investments, to develop huge pharmaceutical markets, and also… to save face! Don't forget the title of the book that the father of AIDS dissent, Peter Duersberg, published in 1996. His title was: "How we INVENT the AIDS virus", in **English** "Inventing the AIDS Virus".

References by Harven E. Viremia in Friend murine leukemia: the electron microscope approach of the problem. Pathology-Biology 1965; 13: 125-134. Sinoussi F et al. Purification and partial differentiation of the particles of murine sarcoma virus (M. MSV) according to their sedimentation rates in sucrose density gradients. Spectra 1973; 4: 237-243. Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature 1970; 226: 1211-1213. Baltimore D. RNA-dependent DNA polymerase. Nature 1970; 226: 1209-1211. Varmus H. Reverse transcription. Sci. Am. 1987; 257: 48-54. Ross J. et al. Murine separation from cellular and murine leukemia virus DNA polymerase. Nature New Biology 1971; 231: 163-167. Beljanski M. In vitro synthesis of DNA on an RNA template by an Esscherichia coli transcriptase. CR Acad. Sci. 1972; 274: 2801-2804. Barré-Siunoussi F. et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired

immune deficiency syndrome (AIDS). Science 1983; 220: 868-871. Panem S. C type virus expression in the placenta. Curr Top Pathol 1979; 66: 175-189. Bess JW et al. Microvesicles are a source of contaminating cellular proteins found in purified HIV-1 preparations. Virology 1997; 230: 134-144. Gluschankof P et al. Cell membrane vesicles are a major contaminant of gradient-enriched human immunodeficiency virus type-1 preparations. Virology 1997; 230: 125-133. Djamel Tahi. Did Montagnier discover HIV? "I repeat, we did not purify!". Continuum 1997; 5: 30-34. Frenchi F. In search of HIV. Strandstrom HV et al. Studies with canine sera that contain antibodies which recognize human immunodeficiency virus structural proteins. Cancer Research 1990; 50: 5628s-5630s. Papadopulos-Eleopulos E et al. Is a positive Western blot proof of HIV infection? Bio / Technology 1993; 11; 696-707. from Harven E. Summary statement. Interim Report of the AIDS advisory panel, Pretoria, May 2000. Published by the South Africa government on April 4, 2001. Urnovitz HB et al. RNAs in the sera of Persian Gulf War veterans have segments homologous to chromosome 22q11.2. Clin Diagn Lab Immunol 1999; 6/3: 330-335. Löwer R et al. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. Proc Natl Acad Sci USA 1996; 93: 5177-5184. Mullis K. "Dancing Naked in the Mine Field". Pantheon, 1998 Gelderblom HR. HIV sequence data base: fine structure of HIV and

SIV. Dourmashkin RR et al. The presence of budding virus-like particles in human lymphoid cells used for HIV cultivation. VIIth International Conference on AIDS. Florence 1997: 122. Weiss R, Wain-Hobson S. The Durban Declaration. Nature 2000; 406: 15-16. Stewart GT et al. Not all accept the Durban Declaration. Nature 2000; 407; 286. Papadopulos-Eleopulos E. A brief history of retroviruses. Continuum 1997; 5: 25-29. Lanka S. HIV; reality or artifact? Continuum 3, # 1,4-9, April-May 1995. Duesberg P. "Inventing the AIDS virus". Regnery Publishing, Inc., Washington, DC 1996.
www.sidasante.com/edh/edhvih.htm

THE PROBLEMS OF ISOLATION OF HIV

Etienne de Harven, MD. Brussels - European Parliament - December 8, 2003

What can we do to better help Africa? What are the priorities that would allow us to control **what is currently described as the AIDS epidemic**? For twenty years, all research has been **based** on the **hypothesis** of HIV. Do we have good reasons today to doubt this hypothesis? Yes, certainly, because big problems persist regarding the isolation **and** purification of HIV. In fact, and despite numerous **claims** to the contrary, this retrovirus has **never** been isolated or purified in a scientifically acceptable manner in classical virology. To properly demonstrate the magnitude of the problem, it is necessary to **compare** the current results obtained with HIV with those obtained,

many years ago, in experimental pathology, with another retrovirus, the **Friend** virus, recognized as being associated with a leukemia in some **mice**. These **two** retroviruses have **very similar** morphologies when examined under the electron microscope, they have identical diameters**???**, and **sediment** at the same density in the sucrose gradients. A direct comparison of the problems posed by their isolation and purification is therefore perfectly appropriate**???**. Mice with Friend's leukemia have a considerable number of retroviral particles in their circulating blood. This phenomenon, which was called "Viremia" in the **past** (1), would be called "Viral Charge" in **today's** language. From a few cubic centimeters of blood **plasma** from these mice, the viral particles were **easily** isolated, by a simple method of ultrafiltration **and** centrifugation.**???** Then prepared for electron microscopy, the results were illustrated as follows: What is truly astounding is that **no one has yet succeeded,** by applying this simple method, in demonstrating the particles of HIV in the blood of any so-called AIDS patient, even if the blood samples are taken. in patients identified, by PCR methods, as having a high "Viral Load"! The **absence** of any data in electron microscopy , making it possible to elucidate the nature of the **so-called** "Viral Load" in AIDS patients, however embarrassing it was, was **underlined** for the **first** time during an important conference on AIDS , in Pretoria, in May 2000 (2). **None** of the experts present at this

conference could demonstrate, or refer to publications in which HIV has been observed **directly in** the blood of AIDS patients. In addition, it will soon be two years since a bonus of 100,000 dollars was officially offered (3) to those who succeed in demonstrating HIV particles in the blood of patients supposed to have a **high viral load.** To date, this bonus has **never** been claimed. Obviously, the isolation and purification of retroviral particles that could so **easily** be done in leukemia **mice** could **never** be done in AIDS **patients**.

ALLEGED ISOLATIONS OF HIV BASED ON NON-SPECIFIC "MARKERS". For 20 years, **medical literature** has been inundated with publications in which the authors have tried to **mask** the absence of retroviral particles in samples taken **directly** from AIDS patients. In all these publications, **molecular** "Markers", **supposed** to be specific for HIV, systematically **replace** the missing viral particles. These markers are **physical**, **biochemical** or **genetic** in nature.

Physical markers. It has been known for a very long time that the retroviruses conventionally isolated from chickens, mice and cats **all have the same** shape and the **same** density, which makes them all sediment at the same level, after sedimentation at high speed in sucrose gradients. In fact, **all** these retroviruses sediment at a density of 1.16 g of sucrose per ml (4). The **so-called** HIV having been **classified as a** retrovirus, we should logically

expect to see it sediment at this **same** density. What we have **also** known for a long time, and **long** before the emergence of AIDS, is that innumerable fragments **and** cellular debris, they too sediment at this **same** density (see 5, 6 for recent confirmation). Harvesting sediment material at this density is therefore by **no** means sufficient proof of the isolation of a retrovirus, **unless** satisfactory controls under the electron microscope**???** make it possible to exclude contamination by cellular debris. This control was and remains essential!**???** And its importance had moreover been emphasized at an **international** conference in **Paris** in 1974 (4). What is very **surprising** is that it is in this **same** laboratory of the Institut Pasteur that, ten years **later**, in 1983, an article was published (7), article in which these controls do **not** appear. However, it would appear (20) that these checks had been **attempted** but that the results were **not** encouraging. And yet it was in this **same** article that the isolation of a retrovirus, the **future** HIV, was **announced**. Unfortunately, it was **this** article that gave AIDS research more than uncertain direction for the next twenty years.

Biological markers

In 1970, Temin (8) and Baltimore (9) discovered **previously unknown enzyme activity in allegedly purified samples of experimental retroviruses.** This enzyme was **called** "reverse transcriptase" because it is capable of inducing**???** DNA synthesis from an RNA

model. This was, indeed, a fundamental discovery that revolutionized molecular genetics. And as this enzyme was observed for the **first** time in samples of carcinogenic RNA viruses ("Oncornavirus"), the idea quickly became established that this enzyme **represented** a **specific** marker for these viruses, hence the decision to give a **new name** for these viruses, the name "Retrovirus". And since then, reverse transcriptase has been considered a **marker** for HIV… And yet, soon after the publications of Temin and Baltimore, it became **clear** that reverse transcriptase was, in fact, a very common phenomenon in biology and was by no means a unique specificity to "Retroviruses" (10, 11, 12). Unfortunately, Temin and Baltimore apparently did **nothing** to verify the purity of the virus samples used in their experiments. Consequently, any **contamination** of these samples by cellular (10), bacterial (11) or mycoplasmatic debris , could just as well account for their observations. In 1983, the Institut Pasteur group **announced** the isolation of a new retrovirus (the **future** HIV), basing their conclusion **mainly** on two criteria, namely 1) the detection of reverse transcriptase activity 2) in sediment material at the density of 1.16 g of sucrose per ml. These two criteria are **meaningless** if they are not checked by electron microscopy**???**, thus excluding any interference by non-viral contaminants, which are known to be very frequently present in large quantities in so-called retrovirus preparations.

Several proteins, **allegedly** of viral origin, are frequently used as **specific** markers for HIV, for example p24. The **most serious doubts** have been expressed on their **specificities** for more than 10 years (15). The **absence** of any correlation between the measurements of p24 and those of the viral load has recently been pointed out (13). Also surprising is the observation made in dogs, indicating that 40% of dogs respond positively in Western blot tests to proteins obtained **by** genetic recombination such as gp120, gp47, p31 and p24 (14). We had to expect such results, because the group from Perth, Australia (Eleni Papadopulos, Val **Turner** and their collaborators) was the first, in 1993, to demonstrate the **complete absence** of specificity of these **so-called** structural proteins of HIV in an article published in Nature / biotechnology (15), a fundamental article which was totally **ignored**. To cite the main examples, gp41 seems to correspond to actin, and gp 120-160 are probably oligomers of gp41. In short, the cellular debris that very often contaminates , poorly purified retroviruses can easily explain the presence of **so-called** retroviral markers, and the **so-called** successes in isolating HIV very probably come **from** a completely **unjustified confidence in markers.** gp41 **appears** to correspond to actin, and gp 120-160 are **thought to be** oligomers of gp41.

Genetic markers **and** measurement of viral load.

This approach could **appear** more attractive for two reasons: 1) it applies directly to the blood of patients, thus avoiding the difficulties of **interpretation** of the data obtained in cell culture, and 2) it is supposed to be quantitative.

However, and as already pointed out, it has **never** been possible to observe particles of HIV in the blood of patients under the electron microscope. What do we measure, then, by the PCR technique? **Most likely** PCR methods amplify small RNA **fragments**, which are more abundant under various conditions of stress **and** chronic conditions (16), and which include retroviral segments derived from endogenous human retroviruses (HERV's). This is not surprising, since about 2% of the human genome has clear?? retroviral homology (17). Consequently, measuring the **alleged** viral load by PCR probably has **no** correlation with a **hypothetical** HIV viremia.

ABUSE OF BEAUTIFUL IMAGES. The "viral load" of newspapers and magazines is enormous, and could be measured by the number of images of HIV that appear almost daily in the world press! These images are very attractive, and frequently high in artificial colors. They illustrate the danger of distorting public information with the graphics that come from our computers. Such images, brought to the attention of the public and the medical

profession, attempt to convey an obvious message: "Yes, HIV has indeed been isolated since it can be portrayed with an electronic microscope"! All these images represent computerized **and** embellished rationalizations **based**, from quite a distance, **on** images of viruses taken with an electron microscope, images similar to that which illustrated, for example, the article by the Institut Pasteur in 1983 (7). But these images **never** come directly from an AIDS patient. They come ALL from complex cell cultures (19), prepared and often exchanged from one laboratory to another, cultures which have been described as true “retrovirus soup” (20), so much had been done to be sure of 'find what we were looking for there. On the other hand, what we apparently **omitted** to do , were the controls , which would have made it possible to clarify the endogenous origin of the viruses observed in the cultures. And even if these checks have been done, their results seem **never** to have been published. We are still waiting for the editor of a newspaper which, alongside beautiful computerized images of HIV, would be **honest** enough to explain to its readers that **such viruses have only been observed in cell cultures and that all of this has yet to be confirmed,** on samples that come **directly** from AIDS patients. The cell cultures used in AIDS research are all **mixed** and highly **stimulated**. Mixed, because they contain for example lymphocytes from a patient, plus H9 cells from Gallo's laboratory, cells well known as chronic carriers of retroviruses (21). Or, as was

the case in the initial observations of the Institut Pasteur in 1983, lymphocytes from a suspected AIDS patient, plus lymphocytes isolated from umbilical cord blood. These lymphocytes originating from the umbilical cord, and therefore being of placental origin, are very likely to be carriers of endogenous retroviruses, the placenta being well known, since 1979, to be a tissue particularly rich in retroviruses (22). In addition, these complex cultures were always **stimulated by multiple growth factors such as phytohemagglutinin, T cell growth factor, or interleukin2, or corticosteroid hormones.** All these factors are known for their capacity to **activate** the expression of endogenous retroviruses (HERVs) which, although defective, can acquire an envelope and bud on the surfaces of cells thus activated. Presumably, this is what happened in 1983 (7) when lymphocytes from the umbilical cord were activated by two of these factors (PHA and TCGF). Unfortunately, the **controls** that would have **verified** this **interpretation** do **not** appear in the literature. In short, electron microscopy has been omitted to exclude the presence of cellular debris in preparations of viruses **wrongly** regarded as purified, and images of viral budding on the surface of placental lymphocytes have been **interpreted dangerously**.

CONCLUSION In conclusion, HIV has **never** been neither isolated nor conclusively purified and that therefore the HIV **hypothesis** of the origin of AIDS must be

fundamentally revised (23, 24, 25, 32). More precisely, without purification of HIV, the specific **antigens** of this virus cannot be rigorously identified (15). And yet these are the antigens that are the **basis** of all the serological tests used today to detect the presence of anti-HIV antibodies, ELISA, Western blots, and more recently **rapid tests such as "Capillus", "Determine", and "Vironostika".** Recombinant DNA techniques, of course, give products of great purity, **but cannot** give them the **missing** specificity. It is therefore **not** surprising that **dozens** of medical conditions, including tuberculosis, malaria, leprosy, multiple blood transfusions, certain vaccines, multiparity, etc., **can all cause** false positive HIV tests (26). There is **no** doubt that retroviral particles have been observed, **not** directly in patients with AIDS, **but** in mixed and highly stimulated cell cultures (7). Most **likely**, these particles represent endogenous retroviruses (17) whose **hypothetical** role in the cause of AIDS has **never** been proven. The HIV particles, **not** found directly in patients, were cleverly **replaced** by “Markers”, because the HIV **hypothesis** had to be saved at all costs (see the Durban Declaration, 27), even at the cost of scientific **integrity** (28). If AIDS was really caused by HIV, how could we understand that after 20 years of intensive research based exclusively on this hypothesis we have **never** succeeded in isolating this virus? Twenty years of research which has not led to any curative treatment, any vaccine, and any verifiable epidemiological prediction ...

It is therefore very urgent to courageously ask the essential question: is the HIV hypothesis correct? Very urgent, because there is a way to see AIDS differently (29), outside the framework of infectious diseases, and outside the framework of retroviruses. And from this perspective, which is full of optimism, the considerable difficulties encountered in efforts to isolate and purify HIV can find a very simple explanation. An explanation that recalls the doubts that many "dissident" scientists have about the **very existence** of HIV. These doubts, which I fully share, are not new and had been clearly expressed many years ago (30, 31). Let us not forget the title of the book published by Peter Duesberg in 1996: "How we invented the AIDS virus" ... Consequently, the priorities for medical assistance to sub-Saharan countries must urgently be revised as follows: 1) Treat all endemic tropical diseases with their specific treatments. 2) **Suspend** all administration of antiretroviral drugs **until** the isolation of HIV and its pathogenicity are scientifically established. 3) **Suspend** the use of serological tests, the **specificity** of which is far from having been demonstrated. 4) Provide the people of Africa with the means to fight against **malnutrition**, as well as a well-controlled distribution of **drinking water,** satisfactory conditions of hygiene and housing, and efficient health infrastructures.

References 1) by Harven E. Viremia in Friend murine leukemia: the electron microscope approach of the problem. Pathology-Biology 1965; 13: 125-134. See also: de Harven E., Pioneer deplores "HIV", Continuum 1997, vol 5 n ° 2, page 24. 2) de Harven E. Summary statement. Interim Report of the Aids Advisory Panel, Pretoria, SA, May 2000. Published by the South African Government, on April 4, 2001. 3)

4) Sinoussi F, et al. Purification and partial differentiation of the particles of murine sarcoma virus (M.MSV) according to their sedimentation rates in sucrose density gradients. Spectra, N ° 4, 1973, pp 239-243. 5) Bess JW et al. Microvesicles are a source of contaminating cellular proteins found in purified HIV-1 preparations. Virology 1997; 203; 134-144. 6) Gluschankof P. et al. Cell membrane vesicles are a major contaminant of gradient-enriched human immuodeficiency virus type-1 preparations. Virology 1997; 230: 125-133. 7) Barré-Sinoussi F. et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983; 220: 868-871. 8) Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature 1970; 226: 1211-1213. 9) Baltimore D. RNA-dependent DNA polymerase. Nature 1970: 1209-1211. 10) Ross J et al. Murine separation from cellular and murine leukemia virus DNA polymerases. Nature New Biology 1971; 231: 163-167. 11) Beljanski M. In

vitro synthesis of DNA on an RNA matrix by an Esscherichia coli transcriptase. CR Acad. Sci 1972; 274: 2801-2804. 12) Varmus H. Reverse transcription. Sci. Am. 1987; 257: 48-54. 13) Franchi F. In search of HIV; 14) Strandstrom HV et al. Studies with canine sera that contain antibodies which recognize human immunodeficiency virus structural proteins. Cancer Research 1990; 50: 5628s-5630s. 15) Papadopulos-Eleopulos E et al. Is a positive Western blot proof of HIV infection? Bio / Technology 1993; 11: 696-707. 16) Urnovitz HB et al. RNAs in the sera of Persian Gulf War veterans have segments homologous to chromosome 22Q11.2. Clin Diagn Lab Immunol 1999; 6/3: 330-335. 17) Löwer R et al. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. Proc Natl Acad Sci USA 1996; 93: 5177-5184. 18) Mullis K. "Dancing naked in the Mind Field". Pantheon, 1998. 19) Gelderblom HR. HIV sequence data base: fine structure of HIV and SIV.. 20) Tahi D. Did Montagnier discover HIV? "I repeat, we did not purify! " Continuum 1997; 5: 30-34. 21) Dourmashkin RR et al. The presence of budding virus-like particles in human lymphoid cells used for HIV cultivation. VIIth International Conference on AIDS. Firenze 1992: 122. 22) Panem S. C type virus expression in the placenta. Curr Top Pathol 1979; 66: 175-189. 23) Shenton J. "Positively False". IB Tauris & Co, publ., London, 1998. 24) Hodgkinson N. "The Failure of Contemporary

Science - How a Virus that Never Was Deceived the World". Fourth Estate Limited, publ., London 1996. 25) Russeil R. “Survey on AIDS - The Truths of the Musles” Editions Vivez Soleil, publ., Chêne-Bourg / Genève, 1996. 26) Johnson C. Whose antibodies are they anyway ? Continuum Sept / Oct. 1996. 27) Weiss R, and Wain-Hobson S. The Durban declaration. Nature 2000; 406: 15-16. 28) Stewart GT et al. Not all accepted the Durban Declaration. Nature 2000; 407: 286. 29) Duesberg P, Köhnlein C and Rasnick D. The chemical bases of the various AIDS epidemics: recreational drugs, anti-viral chemotherapy and malnutrition. J. Biosci. June 2003, 28 # 4, pp 383-412. 30) Papadopulos-Eleopulos E. A brief history of retroviruses. Continuum 1997; 5: 25-29. 31) Lanka S. HIV, reality or artefact? Continuum 1995; 3, # 1, 4-9. 32) Rasnick D. The AIDS Blunder. "Mail & Guardian", Johannesburg, SA, Jaznuary 24, 2001. 33) Duesberg P. "Inventing the AIDS Virus". Regnery Publishing, Inc., Washington, DC 1996 . Etienne de Harven, MD Formerly Member of the Sloan Kettring Institute, New York, NY, Emerit. Teacher. Pathology, Univ. of Toronto,
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https://www.rki.de/EN/Content/Institute/History/history_node_en.html
https://www.sourcewatch.org/index.php/Koch_Brothers

https://en.wikipedia.org/wiki/Robert_Koch
https://en.wikipedia.org/wiki/Koch%27s_postulates
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3916274/
https://www.geni.com/people/Robert-Koch-Nobel-Prize-in-Physiology-or-Medicine-1905/6000000019099551516
https://www.geni.com/people/Johannes-Koch/6000000002940096291
https://www.sourcewatch.org/index.php/Koch_Family_Foundations
https://en.wikipedia.org/wiki/Robert_Koch_Institute
https://www.robert-koch-stiftung.de/index.php?article_id=2&clang=1
https://www.libraryofsocialscience.com/newsletter/posts/2016/2016-07-11-rak-h2.html
https://www.libraryofsocialscience.com/newsletter/posts/2018/2018-12-11-immunology.html
https://www.rki.de/EN/Content/Institute/History/rk_ns_node_en.html
https://timeline.com/the-koch-family-s-nazi-ties-are-more-entrenched-than-you-think-37c645012da0
https://www.algemeiner.com/2020/04/06/hitler-as-plague-doctor/
https://www.npr.org/2016/01/19/463565987/hidden-history-of-koch-brothers-traces-their-childhood-and-political-rise
https://www.nytimes.com/2016/01/12/us/politics/father-of-koch-brothers-helped-build-nazi-oil-refinery-book-says.html

https://www.robert-koch-stiftung.de/index.php?article_id=2&clang=0 https://www.robert-koch-stiftung.de/index.php?article_id=13&clang=1 https://www.robert-koch-stiftung.de/index.php?article_id=12&clang=1 https://www.robert-koch-stiftung.de/index.php?article_id=271&clang=1

Alive & Well $50,000 Fact Finder Award Find One Study, Save Countless Lives

The **missing evidence** we're looking for is a study published in a peer reviewed medical journal that shows the **validation** of any HIV test by the direct isolation of HIV from the fresh, uncultured fluids or tissues of positive testing persons. Since **no** HIV test **directly** detects HIV itself, and since the tests currently used to diagnose HIV infection rely on **surrogate** markers such as antibodies **or** genetic material, a study should exist somewhere in the published medical literature which shows that **at least one type of** surrogate test for HIV has been **validated** for accuracy by the direct isolation of HIV itself from people who test antibody, RNA or DNA positive. The $50,000 offered through Alive & Well will be paid by **two anonymous** donors committed to the possibility of integrity in AIDS science and to creating a world in which no one goes hungry. Award funds will be disbursed within 30 days of presentation of the required

evidence as described above. For each month the award remains uncollected, Alive & Well founder Christine Maggiore, will make a personal donation to Heifer International (http://www.heifer.org) whose work resolves the most prevalent causes of disease and death in Africa: poverty and malnutrition. The Fact Finder Award expires on April 23, 2009, the 25- year anniversary of the historic announcement by the US Department of Health and Human Services **that** HIV had been found and identified as "the **probable** cause of AIDS." Potential participants should note that detection of other surrogate markers not specifically mentioned in this text (reverse transcriptase, p24, etc) or the presence of "retrovirus-like particles" in co-culture do not substitute for evidence of direct isolation of HIV from fresh, uncultured fluids or tissues. Can a study that validates HIV tests really be missing from the medical literature? That's what we want to find out. It,s been 23 years since the discovery of HIV and the development and marketing of the HIV antibody test, yet **no** study ever validated HIV tests by the direct purification of HIV from persons who test positive or have a "viral load." As far as we can tell, the accuracy of the HIV antibody tests used around the world to say someone is infected with HIV has **never** been properly established, and there's **no** information in the published medical literature showing how many positive tests occur in the absence of infection with HIV. What would a validation study prove? The accuracy of an antibody or

other surrogate test for a virus can only be established by verifying that positive results are found only in people who actually have the virus. This standard for determining accuracy was **not** met in 1984 when the **first** HIV antibody test was developed. To **this** day, positive HIV antibody screening tests (ELISAs) are **verified by a second antibody test of unknown accuracy** (HIV Western Blots) **or by** "viral load," **another unvalidated test.** A validation study would prove the ethical and scientific basis for the practice of telling people who test antibody, DNA or RNA positive that they are infected with HIV. **Without** evidence of validation by direct purification of the virus, a diagnosis of HIV infection rests on **unverified beliefs** and **unfounded assumptions.** Is a validation study worth $50,000? To us, $50,000 is a small price to pay for scientific validation that HIV tests give positive results only to people who actually have the virus. Current HIV tests signal the presence of antibodies that react with an assortment of proteins **associated??** with HIV, however, **none** of these proteins are **unique or specific to** HIV. Without a validation study, no honest, well-informed doctor can say with any degree of certainty that someone who tests positive is indeed infected with HIV. **Why** can't "viral load" tests be used to validate HIV antibody tests? Like HIV antibody tests, viral load tests are **not** able to directly detect HIV itself. Instead, these tests detect **only** fragments of genetic material (DNA or RNA) **associated** with HIV. To date, we have not found a

study showing that the DNA or RNA attributed to HIV is found only in people who are actually infected with HIV using direct isolation as a gold standard to determine true infection. In fact, viral load tests carry **disclaimers** stating they are **"not intended to be used as a screening test for** HIV **or as a diagnostic to confirm the presence of** HIV **infection**" (Roche, Amplicor HIV-1 Monitor Test). Why isn't an antibody test that's verified by another antibody test good enough to say someone is infected with HIV? The **rationale** for the use of antibody tests is that the immune system has the ability to detect foreign agents or viruses and to respond by producing antibodies that react with those agents or viruses. However, this rationale does not work in **reverse**. That is, the observation of an antibody reaction with a particular agent or virus does not prove that the antibody was produced in response to that particular agent or virus. The problem with using antibodies **alone** to indicate infection with a particular agent or virus is **two fold**: 1. Antibodies can only be associated with a disease after it is shown that they are consistently generated after exposure to the pure virus. We are **unaware** that this has ever been accomplished with HIV. 2. Antibodies engage in indiscriminate relationships with a variety of agents or viruses. One could say that antibodies are "promiscuous," that is, antibodies meant for one agent or virus may react with another agent or virus that is a perfect stranger. Or, to put it technically, there is **ample** evidence that antibody

molecules, **even the most pure** (monoclonal antibodies) are **not mono-specific, and** that they cross-react with other, non-immunizing antigens. What does all this mean? What this means is that people do not necessarily have the virus that their antibodies may appear to suggest they have. Here are some examples of how **misleading** antibody tests can be: 1. People can have positive antibody responses to certain **laboratory chemicals**, but this does not mean they are infected with laboratory chemicals. 2. People vaccinated for **polio** may test positive for antibodies to polio even though they don,t have polio. 3. People exposed to TB may test antibody positive for TB but this does not necessarily mean they are **currently** infected with TB. 4. The test for glandular fever measures , antibody response to red blood cells of sheep and horses, but a positive test does not mean that someone is infected with sheep or horse blood, or that animal blood causes glandular fever. From these few examples, we understand why antibody response alone cannot determine if someone is infected with a particular virus. What's the **solution** to the problems with HIV antibody tests? Since antibody reactions can come from more than one possible cause, scientists need more information before they can claim that an antibody reaction alone means a person is actually infected with a particular virus. Long **before** the HIV test was introduced into routine clinical practice, scientists needed to prove that a positive test means that HIV itself is present, too.

This is especially important given the profound implications of testing HIV positive. **People,s lives literally depend on the specificity of HIV tests.**

What is specificity? In this case, the formal, mathematical definition of specificity is the number of negative tests in a large group of individuals who do not have HIV infection. If 100% of 1,000 people who do not have HIV infection **also** test antibody negative, the specificity of the antibody test is 100%. If one uninfected person tests antibody positive, the specificity of the test is reduced to 99.9% (999/1000) due to the single false positive result. A high specificity is desired when screening to make sure that very few false positives occur. As far as we know, the specificity of HIV tests has not been established in this very necessary scientific manner.

What is sensitivity? The formal, mathematical definition of sensitivity is the number of positive tests in a large group of individuals who actually do have HIV infection. If 100% of 1,000 people who have HIV infection also test antibody positive, the sensitivity of the antibody test is 100%. If one infected person tests antibody negative, the sensitivity of the test is reduced to 99.9% (999/1000) due to the single false negative result. A high sensitivity is desired when you don't want any gold standard positives to slip through undetected.

Is specificity the same as accuracy? How is the accuracy for an HIV test determined? A study that establishes the sensitivity and specificity of an HIV test would provide a scientific basis for claims of accuracy. Sensitivity + Specificity = Accuracy .

How did AIDS experts **arrive** at the specificity of the HIV antibody tests used today? According to the medical literature on AIDS, the specificity of HIV antibody tests has been evaluated by testing **healthy** individuals such as blood donors. **Because** these individuals are **healthy**, it's **assumed** that negative antibody test results mean they don't have HIV, and because few if any of these people test positive, AIDS experts use this information to **claim** that the antibody tests are highly specific. This evaluation is the **wrong** type of experiment from which to draw such conclusions for two reasons. **First**, healthy people do not have a large number or a variety of antibodies to react with the test, so there are not enough antibodies available to measure the propensity for unwanted reactions. **Second**, good health cannot be used as a substitute measure for the **absence** of HIV infection any more than good health can be used as a substitute measure for the absence of kidney stones, pregnancy, cerebral aneurysms, pathogenic bacteria or coronary artery disease. What is the **correct** solution to the problem of distinguishing who is and who is not HIV infected? According to Dr **Valendar Turner** (http://www.theperthgroup.com), a

medical doctor who has examined the problems with HIV tests, “The **solution** is obvious, scientifically speaking. You have to use HIV itself to validate the tests. To do this, you must take **two samples** from each person in a study and divide the two blood samples from each person in two groups: One sample to test for the antibody reactions and the other to try to **directly isolate** HIV. To know what the HIV antibody tests tell you about HIV infection, you then compare the reactions (positive tests) with what you are trying to find or measure (**actual** virus). The only way to **distinguish** between real reactions and false reactions (crossreactions) is to use **direct** isolation of HIV as an independent yardstick or gold standard.” What would the results of such an isolation experiment show? The results of such an experiment would show how many of an appropriately chosen group people from whom HIV cannot be isolated have a positive antibody reaction anyway. This would tell us how many positive antibody tests occur in the absence of HIV infection. **Without** validation by direct isolation of the virus from the **fresh**, **uncultured** fluids or tissues of people who test positive, AIDS “experts” **cannot** know what positive and negative test results **actually** indicate. That there are **no** data establishing the accuracy of HIV tests , is particularly concerning given that people who test positive are **said to be** infected with a fatal, incurable virus and treated **as if this were an indisputable truth.**

Why is it called a Fact Finder Award? What we want to find meets the dictionary definition of a fact*, which is: 1. Something that can be shown to be true, to exist, or to have happened. 2. The truth or actual existence of something, **as opposed to the supposition** of something or a **belief** about something. 3. A piece of information that shows that **statistics** or statements are true. 4. The circumstances of an event, motion, occurrence, or state of affairs, **rather than an interpretation** of its significance. 5. Something that is based on or concerned with the evidence presented in a legal case. In our search of the published medical literature, we have **not** found evidence showing that popular **interpretations** of the significance or accuracy of HIV tests are scientifically valid or correct. In exchange for this fact, we will award the finder. Hence, the "Fact Finder Award."

If a study that validates HIV tests may **not** exist, what's the point of offering the award? We hope a monetary incentive will motivate someone to find a study we've missed or to inspire a group of AIDS researchers to conduct. The spirit of the Fact Finder Award is win-win. Whether or not the award is ever claimed, everyone would benefit from a forthright and scientific discussion of HIV tests.

www.aliveandwell.org/docs/FactFinderAward.pdf
http://www.altheal.org/isolation/prize.htm

http://www.virusmyth.com/aids/hiv/slartefact.htm
https://en.wikipedia.org/wiki/Ernst_Ruska
https://web.archive.org/web/20070831094917/http://helmut.ruska.de/thelancet/text_e.html
https://www.thetruthbarrier.com/2013/06/20/can-electron-microscopy-resolve-the-hiv-battle-an-exclusive-interview-with-em-pioneer-dr-etienne-de-harven/
https://www.jpands.org/vol15no3/deharven.pdf
www.paganpressbooks.com/jpl/RA-SHORT.HTM
https://www.gov.uk/guidance/high-consequence-infectious-diseases-hcid
https://hub.jhu.edu/2019/11/06/event-201-health-security
https://www.youtube.com/watch?v=t0uemkf9c88ax
https://revealingfraud.com/2020/03/health/35-sources-the-covid19-corona-virus-is-over-hyped-and-likely-fraud/

https://abruptearthchanges.com/2017/11/17/dr-stefan-lanka-the-history-of-the-infection-theory

there is NO virus . people falling sick / dying -- look for the cause somewhere else . susceptibility to seasonal changes in climate , toxic medicines & chemicals , lack of nutrition , chemical / biological warfare , mental stress, vaccines , uncleanliness , and **unknown reasons etc etc ... ... but dont call it virus.**

Medically relevant viruses don't exist. No one has identified , isolated any . see my 2300 pages paper in academia.edu

Coronavirus: a reliable test is badly needed. We don't have one.. By Jerome Burne,

Media coverage of the rapidly growing Coronavirus 2019 nCov epidemic is **unanimous** that official bodies are doing everything possible to contain it, using all the tools of modern medical science and public health resources. The UK government has committed 40 million pounds to research. We are told that this novel virus was rapidly **identified**, a test developed and those testing **positive** are being rapidly quarantined and treated with the latest medications.

But there is a dissenting voice. David Crowe is a Canadian software and telecommunications engineer with a degree in mathematics and biology who has become an **independent expert** in 21st Century global infections such as SARS, Ebola and flu. Working from a database of 10,000 **scientific?** papers, government, corporate and mainstream media reports, he has been raising **fundamental questions** about the way **"viral" epidemics are identified and treated.**

A rush to judgement .

Crowe describes the current response as a **'rush to judgement, based on** the rapid application of an **unproven test, made worse** by the use of **powerful unproven drugs with toxic side-effects on those who test positive.'**

The Chinese seems to have tacitly acknowledged the issue by starting to **change the way diagnoses are recorded**.

Some of the evidence for his claim emerged in the aftermath of the last global epidemic caused by a **coronavirus** known as **SARS** (Severe Acute Respiratory **Syndrome**). It was **first** reported in Asia in February **2003**, spreading to more than two dozen countries around the world but was contained **within** the year.

Out of the 8,098 who caught it, 774 died.

After the epidemic, which triggered much the **same** response as the current emergency, doctors and scientists began publishing **insider** accounts of what had happened in journals that are **rarely** seen by the general public. Some of them concerned the **very toxic drugs** used to treat **SARS** patients.

These studies suggest that in the **early** days, patients with pneumonia were **diagnosed** with **SARS because** the symptoms – fever, headache, an aching body and a dry

cough -were **similar** to those of pneumonia and flu. But the drugs they were given were **much more toxic** than those used for pneumonia, which could be why **SARS** gained the reputation for being such a **deadly disease.**

At least some of the patients **died from the treatment, not from the disease.**

Damage to blood cells and the liver.
For example, a report commissioned by a World Health Organization expert panel concluded that the antiviral drug **ribavirin**, widely used during the epidemic, caused the **destruction** of **red blood cells** (hemolytic anaemia) in one-third to **two-thirds** of patients and that 75% of them developed **liver** problems. The drug is also known to cause **'flu-like** symptoms such as fever, difficulty breathing, body aches and pains as well as being able to trigger **psychiatric** conditions such as **depression**, **psychosis** and **aggressive** behaviour. Other reports showed that **high dose corticosteroids**, also **widely** used, caused lasting side effects, most notably **serious neurological** and **bone** problems. There is also **evidence** that these drugs, with their extremely unpleasant side effects, contributed to their **deaths**. Crowe's research found that in the countries most affected by SARS, the **rich ones** – Singapore, Hong Kong and Canada – **had a higher death rate than the poorer countries** – China and Vietnam. This, he suggests, could have been due to

high doses of the more expensive **injectable** ribavirin being used in the rich countries, while cheaper, low-dose, **oral** ribavirin was often used in poorer countries. SARS was **so feared, not just because it was thought to be more deadly than other respiratory diseases, but also because it was believed to be highly infectious – same with coronavirus .**

But Crowe has evidence that this was a **mistake** too. It certainly **doesn't** fit with an accidental experiment carried out at a Chinese hospital that **mistakenly** placed SARS and AIDS patients on the same floor of a hospital. The vulnerable patients who escaped being infected .The AIDS patients were suffering from **significant immune suppression & they intermingled for several weeks** with the **supposedly highly infectious SARS** patients.

Yet not a single AIDS patient got SARS, **not even the one** AIDS patient who was put in a room with SARS patients.

The idea that SARS was highly infectious **was due to the official definition of the disease.** To be diagnosed with SARS you had to have had **contact** with another victim. So, SARS patients always had **proven** contact with another SARS patient , was , **because the definition demanded it.**

But his research, which also involves **other scientists critical of the theory that** HIV causes AIDS, **has identified a more fundamental problem – how the virus causing an epidemic is identified?**

Although media and many "scientific" papers make it sound as if the test being used can detect the latest virus.

What the test is **actually** looking for is a particular strand of "RNA" which, it is **assumed**, comes from the **new** "virus". The test then makes another **assumption** that the RNA/virus combination is **always** the **cause** of the illness when it is found in a sick person. **No** symptoms? You can **still** test positive for the "virus" . The **basic rules** for proving disease **causation** are known as Koch's Postulates (after the 19th Century bacteriologist Robert Koch / of **Koch Brothers / supporters of Hitler & Mussolini**) and they demand that a disease-causing entity, such as a **virus**, is **purified** as a first step. But this has **not** been done, as the authors of a recent paper **admitted**: **'"we did not perform tests for detecting the infectious virus in blood".** If a virus is the **cause** of an infection, then it should be able to cause disease by **itself**. But there are plenty of reports where this **doesn't** happen. For example, in **one** family the boy, who had no symptoms, tested positive while his mother, who was quite ill, was tested **18** times but found to be negative each time. **Another** study reported that four Germans

tested positive after meeting a Shanghai-based woman in Germany, who became sick on her way home, showed **no** subsequent signs of "severe clinical illness."

False positives are dangerously misleading. For instance, even if an epidemic began to die out, public health officials would **still** be getting positive results from an **unreliable** test and insist that the epidemic was **still** a threat.

Testing all of Wuhan's 10 million inhabitants with a 99% accurate test would give you 100,000 false positives.

One simple way to establish the false positive **rate** would `be to test at least a thousand healthy people, without **symptoms**, **outside** the epidemic zone to find out how many tested positive.

However, no serious attempts to establish true or false positive rates have been published. But the Chinese government have just changed the way new cases are recorded, according to a tweet from a Hong Kong journalist. The original WHO guidelines for diagnosing 2019 Coronavirus said that a **positive** test was **all** that was needed. The person **didn't** need to have **symptoms** or to have had recent contact with someone who was infected. **Now** cases that were diagnosed

without symptoms are being **removed** from the record of new cases if they don't develop them.

A recognition of the **failings of the test** that should make infection figures more realistic.

Drugs used in epidemics are heavyweight and untested. Doctors who **"believe"** that they have a deadly new viral epidemic on their hands will almost always reach for the **most** potent medicines in the cabinet. Often these medicines have **not been used much before, or only used for different diseases, and it is impossible to get good scientific data from** this situation , where denying patients "life-saving" medications to give them a placebo would be considered unethical. But how can anyone know that drugs **never** before used for a condition **are** "life-saving" and **not** outrageously dangerous? The epidemic is following this pattern.

In the first major survey of 41 coronavirus patients, **all** were given antibiotics **(not effective against "viruses"**), and 93% the "antiviral" drug oseltamivir (**Tamiflu**). Corticosteroids were given to 22% and some were given **invasive** respiratory assistance, which was also associated with lasting problems during **SARS**.

A **second** survey published 6 days later reported that **fewer** patients were given "antivirals" but there were

more types being used. As well as oseltamivir were three, known to have a wide range of **toxic** effects – ganciclovir, lopinavir and ritonavir. There is an **extensive** literature on **oseltamivir**, which is summed up by Canadian drug policy researcher **Alan Cassels,** in a recent tweet, **"It's a useless drug".** Ganciclovir & many AIDS drugs are highly toxic. It causes serious **anaemias** and has been shown to cause cancer and mutations in animals. The AIDS drugs **Lopinavir** and **Ritonavir** have long been packaged in **one** pill together as Kaletra: its major side effects are listed as **pancreas failure, liver toxicity, diabetes and** redistribution of body fat. The patients in **China** are already **older** and **frailer** than average. and 51% had **pre-existing** conditions, such as **heart disease, diabetes, respiratory system diseases** and **cancer** or **nervous system diseases.** These are precisely the people who can**not** withstand "antiviral" drugs and corticosteroids.

But those who have died, and will die, are all being classified as coronavirus deaths.

Apart from encouraging the use of drugs with **toxic** effects, the pandemic panic will almost certainly generate permission to **test** and approve vaccines for the "virus", especially if the **faulty** testing continues, and more and more cases are diagnosed. Given the **relatively small number of patients,** even in this pandemic, the use of

pharmaceutical drugs is not a big money-maker, but it is certainly good **publicity**, the big money will be in a vaccine that can be given to millions, or even billions of people.

And that will give power to the medical cartel to invade into the human rights & political control.

Treatment is worse than the infection.

---- **Viruses** were claimed to exist and to be the cause of disease through **negative** evidence. When materials could be forced through a filter so fine that bacteria could not get through, yet still cause disease when injected into an animal (often directly into the **brain**), it was **claimed** that a "filterable virus" was present.

- I am not convinced , what electrons are , how that can be identified .Its purely theoretical. I suppose, electron microscope is just fiction .

Prior to the invention of the **Electron** Microscope in the 1930s it was **not** possible to see particles this small. With the electron microscope the new breed of virologists started to look at impure materials and claim that they could spot the viruses. The problem is that just by looking at a particle you **cannot** tell what it is or what it does without fulfilling Koch's postulates.

Koch's postulates were developed by the 18th century German bacteriologist Robert Koch. Stated simply:
Purify the virus (you can use an electron microscope to verify that your sample is pure – all particles should look very **similar**).
Inject the virus into a vulnerable animal.
Verify that the symptoms of the disease arise.
Repurify the virus.

It is very important to note that these are **logical** postulates, **not** scientific laws. They are, in other words, just simple, straightforward, every day logic. Koch put them into simple words and forced those who promoted infectious disease theories (most of which were **wrong**) into a corner. Fulfill these simple logical postulates, or go home. **(only big players could survive .)**

The problem for viruses was that, even into the electron microscope era, a **top** virologist was **forced** to admit that, **"It is obvious that Koch's postulates have not been satisfied in viral diseases"** (Rivers TM. Viruses and Koch's Postulates. J Bacteriol. 1937 Jan; 33(1): 1-12).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC545348/pdf/jbacter00773-0005.pdf
https://davidcrowe.ca/SciHealthEnv/papers/5393-Viruses-Koch'sPostulates.pdf

So, instead of satisfying these simple logical postulates, he just proposed **two new ones.**
A **specific** virus must be found associated with a disease with a degree of regularity.
The virus must be shown to occur in the sick individual **not** as an incidental or accidental finding but as the **cause** of the disease under investigation.
These are obvious **nonsense**, but even today this paper is still referenced as seminal, and virologists spend more effort trying to rewrite Koch's postulates than to fulfil them.
It helps to know a little bit about **how** virologists work, **getting past all** the high-tech, sophisticated equipment, and into the logic. This is the approximate **sequence** of events, you will find **repeated** thousands of times in the virology literature:
Get a sample from a diseased person or animal. Blood, semen, urine.
Purify it a little bit (e.g. **spin the blood to get the serum**).
This is called the 'isolate', even though nothing has been isolated.
Possibly put it through a **filter** (to remove bacteria and whole cells).
Add to a culture of cancerous cells (often the HeLa from a woman with cancer **or** Vero cells from monkeys).
Add a number of **stimulating** and **toxic** chemicals, as well as nutrients for the cells.
Leave for a while (a week or two).

Check for **signs** of a virus including:
Particles of the expected size and shape under an electron microscope.

[[VIRUSES AND KOCH'S POSTULATES1 THOMAS M. RIVERS .. 1936 (edited out unsubstantiated claims)
From The **Rockefeller** Institute for Medical Research, New York .

Diseases at one time were thought to be caused by wrath of the gods, configuration of stars or miasmas. After a real struggle that occurred not so many years ago, certain maladies were shown to be induced either by small animals or minute plants, e.g., protozoa, fungi, bacteria and spirochetes. Indeed, the victory was so great that most workers in time began to consider that all infectious diseases, including those whose incitants had **not** been discovered, must be caused by agents **similar** to those already recognized. According to them, there could be **no** infections that were **not** caused by protozoa, fungi, bacteria or spirochetes, and to intimate that some infectious agents might be inanimate constituted heresy of the first order. Even at the present time, the cause of certain diseases is said by some individuals to be **unknown** or undiscovered, because no cultivable bacterium or visible protozoan parasite of etiological significance has been demonstrated in them. For instance, **a few years ago** Cowie made the statement in a scientific

paper that the **etiological** agent of **poliomyelitis** is **unknown**, and in the recent **book**, An American Doctor's Odyssey, Heiser remarked that **"the microbe which causes smallpox has never been discovered."**

In spite of the general acceptance of the idea that **all** infectious diseases are caused by protozoa, fungi, bacteria or spirochetes, some workers have always contended that there might exist **other** infectious agents **in**capable of **classification** with those already known. Furthermore, very early in the bacteriological era a few discerning individuals appreciated the fact that there was **no** reason, **except** analogy, for assuming that **all** infectious agents must be living autonomous organisms.

Through the activities of these investigators a group of disease-producing agents, known as **viruses**, has gradually become **recognized**. The **exact nature of these agents is not known;** some may be the midgets of the microbial universe, others may represent forms of life **unfamiliar** to us, while still others may be **in**animate incitants of disease.

Regardless of **lack** of complete knowledge of their nature, it is decidedly **in**correct to say that these agents are **un**known. **???**

As early as **1840**, before the specific relation of microorganisms to disease was accepted, **Jacob Henle** stated the conditions that should be met before an agent could be considered the proven **cause** of an infectious malady. Unfortunately, investigators were **not** guided by Henle's remarks, and it was necessary for **Robert Koch to restate and** emphasize them 40 years later.

In an article on the etiology of tuberculosis Koch in 1884 made the following statement**:** The facts obtained in this manner can in every possible way **serve as proof** to which only **extreme** skepticism can still raise the objection that the organisms found are **not** the cause **but only** concurrent phenomena of the disease. To be sure this **objection often has a real justification and** therefore it is not sufficient to establish only the concomitant occurrence of disease **and** parasite but the parasite **must be shown to be the real cause.** This can be done **only by fully isolating** the parasite from the body and all products of disease which might be considered as having a deleterious effect and producing the disease **again with all its characteristics by** the introduction of the isolated organisms into a normal host.

In 1890, speaking of bacteriological research before the Tenth International Congress of Medicine in Berlin, Koch expressed the same ideas in the following **less mandatory** manner: However, if it can be proved: **firstly** that the

parasite occurs in **every** case of the disease in question, and under circumstances which can account for the pathological changes and clinical course of the disease; **secondly**, that it occurs in **no other** disease as a fortuitous and nonpathogenic parasite; and **thirdly**, that it, after being fully isolated from the body and repeatedly grown in **pure** culture?, can induce the disease anew; then the occurrence of the parasite in the disease can no longer be accidental, but in this case no other relation between it and the disease **except** that the parasite is the **cause** of the disease can be considered.

The above conditions laid down for the proof of the etiological relation of a microorganism to a disease constitute **what are now** known as Koch's postulates. His dictum has had a profound influence on workers investigating infectious maladies and for many years an infectious agent was **not** accepted as the cause of a disease unless the postulates had been satisfied.

There are certain workers who still **refuse** to agree that the **cause** of an infectious disease has been discovered **unless all** the conditions originally laid down by Koch have been met.

Koch himself quickly realized that in certain instances all the conditions could not be met, and in his paper before the Tenth International Congress of Medicine (1891) ….

we are justified in stating that if only the first two conditions of the rules of proof are fulfilled, i.e., if the regular and exclusive occurrence of the parasite is demonstrated, the **causal** relationship between parasite and disease is validly established.
(Robert Koch from the family of **Koch Brothers**. Its not sufficient , if he asserts something . If his postulates are accepted as guidelines , at some point in time , it doesn't mean we need to /have to follow , whatever he comes up with).

At the time when they were formulated Koch's postulates were essential **(not really)** for the progress of knowledge of infectious diseases; but progress having left behind old rules requires new ones which some day without doubt will **also** be declared obsolete. **(new rules must be logical scientific sensible - who make these rules also matters).**

Thus, in regard to certain diseases, particularly those caused by viruses, the blind adherence to Koch's postulates may act as a hindrance instead of an aid **(what is a virus).**

For instance, the idea that an infectious malady can be caused only by the action of a single agent is incorrect.

Koch certainly did not have tissue-culture methods in mind when he proposed his rules of proof.

Koch's postulates are responsible for some odd conclusions regarding the cause of certain “viral” maladies. For example, a few investigators have claimed that **streptococci** are the inciting agent of **poliomyelitis**. Such claims, according to them, **are based on the fact that Koch's rules have been satisfied.** That is, streptococci have been found associated with the disease, they have been obtained in pure cultures from patients with the malady, they produce **paralysis** when injected into monkeys and rabbits, and they have been recovered in pure**??** cultures from the experimental hosts.

Furthermore, individuals **recovering** from poliomyelitis possess antibodies**??** against the streptococci. To those unacquainted with the viral field and particularly to clinicians and bacteriologists unfamiliar with the pathological picture of poliomyelitis, these claims seem valid. Consequently, they wonder why streptococci are not more generally accepted as the cause of infantile paralysis. The reason for lack of general acceptance is a simple one; the disease **produced in the experimental animals is not poliomyelitis.** Paralysis is **not** a characteristic sign of a **single** disease, and the pathological picture observed in the experimental hosts is

quite different from that seen in human beings dead of **infantile** paralysis.

It is obvious that Koch's postulates have not been satisfied in viral diseases. Moreover, it is equally evident that proof of the etiological significance of **viruses has been obtained without their satisfaction.**??

Such a statement, however, does **not** imply that certain conditons do not have to be met before the specific relation of a virus to a disease is established. The conditions are: (a) A **specific virus must be found** associated with a disease with a degree of regularity. (b) The virus must be shown to occur in the sick individual **not** as an incidental or accidental finding but as the cause of the disease under investigation. In many respects the conditions just stated for viral maladies are **similar** to those of Koch for the proof of the specific relation of bacteria to disease. Nevertheless, there are certain **differences**. In the **first** place, it is **not obligatory to demonstrate the presence of a virus in every case of the disease produced by it.!!!!!!!!!!???**
Secondly, the existence of virus **carriers** is recognized. **Finally**, it is **not** essential that a virus be grown on lifeless media **or** in modified tissue cultures.

How does one go about **proving** that a virus is the **cause** of a disease? Viruses, regardless of whether they are

parasites or the fabrications of autocatalytic processes, are intimately associated with **host cells** and, therefore, should always be found **at the proper time??** in **specific??** lesions.

In addition, viruses, as is the case with bacteria, may be found **also in the blood stream, not** necessarily multiplying there **but** appearing frequently **only** as a phenomenon of **overflow** from lesions in the tissues. With these facts in mind, **tissues with** lesions**, exudate** from such lesions, and **blood** are collected aseptically and **inoculated** into a susceptible experimental host of the same **or** different species. **The material should be free from ordinary microbes; if not, the microbes should be killed or removed in a proper manner,** e.g., by filtration**. (filtration kills microbes ??)**. If the inoculated animals become **sick** or die in a characteristic manner, and, if the disease in them can be transmitted from animal to animal by means of **inoculations** with blood **or** emulsions of involved tissues **free** from ordinary microbes or rickettsiae, **one is fairly confident??** that the malady in the experimental animals is induced by a virus **(reductionism to the extreme !!!!! exudate** = a mass of cells and fluid that has seeped out of blood vessels or an organ, especially **in inflammation. Something caused** inflammation , and that poisoned material is injected into a normal host , and the host is made sick / killed . **proof enough of the cause ? definitely not)**.

On the other hand, such findings do **not** necessarily indicate that the active agent was **present** in the original material **used** for inoculation of experimental hosts. When a **natural disease?** under investigation exhibits characteristic features, e.g., paralysis or intracellular inclusions, they are sought for in the experimental malady. If one finds them, one is encouraged, **but proof is still lacking** , that the virus operating in the experimental hosts was present in the material taken from the individual with the **natural?** infection. **Not infrequently , several** "viruses" produce the **same** clinical and pathological pictures, and at times the **same** virus does **not** induce **similar** changes **in different** hosts. Consequently, **regardless** of the disease picture produced in the experimental animals, **one is still faced with** the problem of demonstrating that the "virus" causing it was present in the material used for inoculation of the **first** group of animals. Experimental animals are subject to "viral" diseases of **their own** which may be encountered with sufficient frequency to cause mistakes.

The "virus" was activated by the experimental procedures. Actually Poison was created in experimental procedures.

Another example of the necessity of proving that , a virus comes from a certain source , is that encountered in our recent work on lymphocytic choriomeningitis. In this

instance, the problem arose **because** the “virus”, with which we were working and **which we believed came from sick human beings, is frequently found in mice under natural conditions.** Furthermore, monkeys and guinea pigs are occasionally naturally “infected”. We were able, however, to show that our stock mice were “entirely free” from infection with this active agent, and it immediately became highly **probable** that we had actually **isolated** our virus from patients. In addition to the fact that **animals** are subject to their own viral diseases which sometimes lead to confusion in the course of experimental work, they may become accidentally contaminated with an alien virus being studied in the laboratory to which they are susceptible. For instance, rabbits are highly susceptible to vaccine virus, and, if in this host serial testicular or cerebral passages, initiated by sterile broth, are made **in** a laboratory where the active agent is under investigation, it is almost impossible to avoid picking up the virus. This fact, which I have demonstrated more than once for my own satisfaction, **most likely** accounts for the ease with which certain Japanese workers **seem to have isolated** from human beings **what they consider the specific** viruses of varicella, measles and scarlet fever. In any event, the **descriptions of** the actions of their viruses and the intracellular changes observed in tissues infected with them are what one would **expect** to find as the result of a vaccinal infection.

Having demonstrated that a “virus” was obtained **from an individual ill of** a certain disease, one **must then prove that the agent was actually causing** the malady instead of occurring fortuitously or instead of inducing a complicating or coexisting infection.

Most investigators are now of the opinion that Levaditi's virus or herpetic virus **is not the cause of** epidemic encephalitis, **even though** it has been recovered occasionally from the brain or spinal fluid of patients with the malady.

Knowledge regarding the regularity with which a virus is associated with a disease may be **highly** important, but information concerning the presence of **antibodies** against the agent and **the time of** their appearance in the serum of patients is **equally** important as evidence of etiological significance of the virus. At the present time **neutralizing antibodies** are the **most** important, but complement-fixing antibodies, agglutinins and precipitins are being recognized more frequently in certain viral diseases and may eventually **assume** a significant place in experimental work on viruses. Under at least two sets of conditions a **virus of no** etiological significance in certain diseases may occur in patients suffering from them.

First, patients who have been affected previously by a viral disease continue as carriers **after recovery** to harbor

the agent. Under such conditions they would **possess** antibodies against this virus at the beginning of their **new** illness as well as during convalescence. **Secondly**, it is conceivable that a virus might gain entrance into an individual and remain there **only a short time causing little or no** reaction. Under these circumstances, the virus, **although** capable of causing disease in experimental **animals**, would **not** incite the production of antibodies in the patients with the result that their serum would be devoid of antibodies **both** at the beginning and end of their illness. Some may doubt that this state of affairs occurs naturally. Nevertheless, it has been encountered not infrequently in experimental work.

If a virus is the actual cause of a disease, immune substances are usually **absent** from the patients' serum at the onset of illness and make their appearance during the period of **recovery**. However, this is **not** universally true, in asmuch as **recovery** sometimes takes place **without** the development of antibodies, and occasionally an individual possessing antibodies against a virus **succumbs** to a disease caused by it. Although the **absence** of antibodies for a virus at the onset of an illness and their appearance later in the course of the disease or during convalescence constitute **highly** suggestive evidence that the virus is responsible for the malady, they **alone** should **not** be accepted as incontrovertible proof that such is the case.**]]**

Detection of proteins **believed to be from** the virus under investigation (but **without** purification, how could one know , what the proteins that makeup the physical structure of the virus are?).

Detection of "DNA or RNA" believed to be from the virus (but again, without purification, how could one know what the "DNA or RNA" inside the virus is?).

Indirect detection of **Reverse Transcription,** the process of converting RNA into DNA , even though it is known this "occurs" in cells (**particularly in the artificial environment of a cell culture**) **without** viruses present. Detection of anything **un**usual, such as giant cells, known as synctitia, even though these anomalies are **never** found in a **living** organism.
(reverse transcription = **unproven** – Stefan Lanka)

On this basis virologists **claim 'isolation' of a virus, even though they have not logically proven that a virus is present, let alone a specific virus.**

If anyone believes that Koch's postulates have been fulfilled for any virus, I would love to hear from you.

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http://healthinsightuk.org/2020/02/12/coronavirus-a-reliable-test-is-badly-needed-we-dont-have-one/

Coronavirus: Low Accuracy Rate of Tests, but maximum Global Government Control

BY SACHA DOBLER ON 4. MARCH 2020 •

First of all, don't panic. If you get tested positive for **COVID-**19, you might as well ask to get a second test. Most governments and the **U.N.have already demonstrated that they cannot be trusted with handling this crisis, whether it is due to incompetence, wilful neglect or worse.** The New York Times reported: **The true death rate could turn out to be similar to that of a severe seasonal flu, below 1%,** according to an editorial published in the journal by Dr. Anthony S. Fauci and Dr. H. Clifford Lane, of the National Institute of Allergy and Infectious Diseases, and Dr. Robert R. Redfield, director of the Centers for Disease Control and Prevention. **Deep concerns have been raised over the corona virus test accuracy,** here I break down why. In south China "The accuracy rate of the test is only 30 to 50 per cent", said Wang Chen, president of the Chinese Academy of Medical Sciences, during a CCTV interview on Wednesday. This means mathematically, if any person is tested three times, they will almost certainly (93%) get at least one positive result, whether they have a corona virus or not. And that's for China, a totalitarian

communist regime with endless human rights violations and without freedom of the press, so we cannot trust anything that comes from the Chinese government. But it must be much better in the West, right? We are not saying that the corona virus does not exist. Generally, for laboratory testing for any disease, a 95% accuracy rate is considered a very reliable test. When health experts say: a given virus tests has, for instance, an accuracy rate of 95%, then people think: aha, a positive test result means they have the virus with a probability of 95% (almost certain). In reality, that's not how it works at all, It depends on how prevalent the virus is in the population and how many people get tested. Let's do some math: A general example (applicable to any disease): assume in a certain population, 1 percent has a certain disease, so out of 100,000 people, 1000 have the disease. If all 100,000 get tested, then 5000 people (5%) get a false positive. That means when a tested person gets a positive test result, that doesn't mean they have a 95% probability of actually having the disease, but in fact, they have a 16.7% probability of having it or an 83.3% probability of not having it (5/6). This is because 1000 real positives + 5000 false positives = 6000 positives.That's 6000 positives of which only 1000 have the disease. 5/6 = 83.3%. So, the more people get tested, the more false positives will emerge. What's worse, if someone gets tested several times, the probability of an individual false positive increases incrementally. If the accuracy rate of a test is

95% and a person goes to a preliminary testing 10 times, then the probability is 40 % to get at least one false positive test result, whether they have anything or not. 1 - (0.95 to the power of 10). =1 -0.5987 =0.4013 =40.13% Almost like flipping a coin. If the accuracy rate of a test is only 90% and a person goes to a preliminary testing 10 times, then the probability is 75 % for at least one false positive test result. For serious diseases, a false positive test can have serious consequences, because of the Nocebo effect (opposite of placebo). The acute fear of death after a deadly diagnosis itself can kill. With that let's return to the current Corona virus: Most Corona fatalities had a preexisting condition. When they die AND tested positive for corona as well, they are counted as corona fatalities and nobody asks for the actual cause of death. As a matter of fact "Chinese officials have unearthed cases of people testing negative six times already before the seventh test confirmed they indeed are positive." This has also been the case in Thailand and Singapore. Neither the WHO nor any western government give an accuracy rate for the novel SarsCoV2 virus test. Health experts urge "Coronavirus: a reliable test is badly needed. We don't have one" As mentioned above, the only number available is the one from south China, where "The accuracy rate of the test is only 30 to 50 per cent", according to Wang Chen, president of the Chinese Academy of Medical Sciences. In other words: people tested positive in south china have a 50 – 70 percent

probability of not having the corona virus. This means if any person get's tested 3 times in china, they will almost certainly get at least one positive result, whether they have a corona virus or not. If we take the middle value of a 40% accuracy rate, if a person in south China gets tested 3 times, then we take: 1- (0.4 to the power of 3) =1-0.064 =0.936 =93.6% probability of at least one false positive result, whether they have anything or not. And this is how the numbers of the WHO are generated and policies are made according to these numbers. Now, when, in a flu season, lots of people have flu-like symptoms and lots of people get tested for this novel virus, there will inevitably be lots of positive test results. What is certain: Whatever people believe the corona virus is or isn't, what is very real and undeniable, are the solutions being enforced by governments, the U.N./ WHO, globalist interests, media and so on. Human rights and constitutional rights within nations are being suspended globally: -Freedom of movement is restricted arbitrarily. Forced quarantines and travel restrictions are sometimes implemented and sometimes they aren't, all depending on incompetent governments. At first, there were no travel restrictions, then they returned corona victims in airplanes together with the general public. Then they locked down entire cities, at the same time they keep importing millions of illegal migrants from other continents without checks. In Germany, people with suspected corona get dragged off public trains. -Freedom of assembly, mass public events

(or any events) are cancelled. -Freedom of commerce is restricted. Cash is being destroyed. Recommended measures include: -Vaccination for lung diseases etc. “Although these vaccines are not effective against 2019-nCoV, vaccination against respiratory illnesses is highly recommended to protect your health.” -Freedom of speech /press is being restricted; monopolisation of information, under the pretence of “combating mis- and disinformation”. The Johns Hopkins Center for Health Security, the World Economic Forum, and the Bill & Melinda Gates Foundation just happened to run a simulation for the exact same pandemic a few month in advance. They jointly propose the following: “Governments and the private sector should assign a greater priority to developing methods **to combat mis- and disinformation prior to the next pandemic response.” (meaning more censorship).** Further announced and promoted are: –forced medication;and once it is available, forced corona vaccinations. – government issued food rationing -government issued mandatory biometric ID to register mandatory vaccines ID2020 and partners launch program to provide digital ID with vaccines In summery: **Even though diagnostic methods are ambiguous, human rights and constitutional rights are being suspended globally** as we speak and more is announced. This just happens to sound a bit like what tin foil hat conspiracy theorists have been warning about. Global government suspending all

rights and taking control over every aspect of everyones life. At some point it will matter little whether someone deliberately helped bring about this crisis, whether a virus escaped from the Wuhan bioweapons lab, or whether it's just a bad flue season, or the problem was accelerated by chemical and electromagnetic influences. The masses have been going along with everything and are now tattling on those who question the authorities. At any rate, don't panic, stay healthy, just in case, wash your hands, stay away from unhinged people, and take your multi-mineral/vitamin supplements with some optional extra vitamin C. As always, have food and water for a few weeks and a plan B at hand.

This man in forced quarantine has only symptoms of a light flu, while his family at home is receiving death threats. He is being tested with the before-mentioned Corona tests. He has to come up with three negative results in a row, in order to be release from the hospital special unit. Statistically, he could theoretically have no virus but always test positive in one out of these 3 tests, and he can be detained indefinitely.
https://abruptearthchanges.com/2020/03/04/coronavirus-low-accuracy-rate-of-tests-but-high-global-government-control/

Dr. Stefan Lanka: The history of the infection theory. (English transcript) BY SACHA DOBLER ON 17. NOVEMBER 2017 •

My name is Stefan Lanka, I am a biologist and virologist. I discovered the first virus, which was in the ocean. That's how I became involved in this matter. First, I recognized that this virus doesn't cause any harm. Secondly, the **Austrian professor Fritz Pol** alerted me to the fact, that **something was wrong with the entire AIDS affair** and the virus might **not** even exist at all. **I checked this and realized, that was indeed the case.** I thought this couldn't be and I remained silent for half a year, for I assumed, I misunderstand something. I couldn't imagine that the entire world would go along with this.

2:00 Then I started researching and became involved in the **infection theory. I realized that everything was wrong**, it initially started with an error, that then turned into fraud, political fraud already under **Otto von Bismarck**, this can easily be proven and reconstructed. This **fraudulent** concept was abandoned after World War II, then **reestablished** by the Americans in order to provoke **fear** and to conduct **population control.**

2:45 Further, I recognized, as I will demonstrate here, **how the infection theory gave rise to the gene theory and the so-called molecular technology and gene manipulation and -technology.** Today's model of the infection theory is used in the form of vaccines, of **fear** from material contagion, in the form of **pandemics**, just as in the current one, which is predicted to erupt any moment, or it will erupt in the second phase or even later and that it will then be encountered with the drug called **Tamiflu**. Incidentally, Tamiflu stands for **toxic** amiflue, there was an apparent glitch in the **naming** process of this chemo-therapeutic drug.

03:32 I'm going to tell the story, how everything developed, in order for you to comprehend, how an error turned into a fraud, a fraud turned into a crime, and how through the **industrialization of this crime,** the madness developed, a kind of madness that endangers all of us, the entire human race.

04:03 We begin at a point in history of ancient Greece there this concept infection developed. It is the **basis** of today's system of medicine that is ruled by systematic fighting and poisoning. It is also why we are supposed to swallow Tamiflu even preemptively and, after the **pandemic** is declared, it should be taken in **large** quantities.

04:55 Before we delve into ancient Greece: **Goethe** tried to warn of the 2006 influence pandemic, **and** also of AIDS and of the swallowing of Tamiflu. He did this in Faust I, **not** in the first edition, that went through censorship, not in the second, but in the **third** or fourth edition he included this essential passage, in which he describes **how even back then a single doctor killed thousands of people:** First he describes in alchemist's language, how the Tamiflu **and** chemotherapy of his time was produced: organic **mercury** compounds, mixed with sirup, Latwergen stands for sirup mixtures, and these will be the hellish Latwergen in alchemistic language and how they were produced. Here are the important lines: Here was the medicine: the patients **died**. **No one asked: who recovered?** (Here was the Tamiflu, the patients died (will die?) And no one asked: who recovered? **No one asked: is the theory correct? Does the virus exist at all?**) So we roamed, with our hellish pills, Among the valleys and the hills, Worse than the pestilence (**plague**) itself we were. And we'll treat the black plague also, a **political** disease just as **AIDS**, influenza, **SARS, BSE,** just as **small pox,** the **collective term of leprosy, from which black plague was derived,** and **also the collective term of polio.** I myself have poisoned thousands: that's quite clear: And now from the withered old must hear **How men praise shameless murderers.**

Goethe. He has **not been taken seriously** 'till today. Whenever there is a tsunami or an earthquake, I think to

myself: Goethe is speaking again. For he warned and this warning was dismissed.

06:49 How did it get to this, what is the mistake, **the overall false assumption?** The general false assumption, **on which the entire western** academic medicine is based on, is this: In the frameworks of the doctrine of juices, it was believed that disease was brought about by an imbalance of juices or fluids in the body. We have many different fluids in the body, about **270** different types, in the joints, in the eye, sweat glands, digestive glands, fluids of the inner ear, brain fluids, spinal fluids and so on. It was **assumed** that a non-equilibrium of juices would **lead to** the development of disease-causing toxins. And it was **believed** from experience, that the administration of **small** amounts of poisons would cause in the body the reaction of production of an **antidote, anti-poison.** This idea derived **from** the experience with the cell toxin **alcohol**. Consumed in small quantities it can be fun, it can also diminish anxieties and the like, but if a young person who never had been in contact with alcohol, drinks half a bottle of liquor, when he is paralyzed enough to not be able to spit, then his stomach must be pumped empty, otherwise he **dies** of alcohol poisoning. **Jelzin** and others, they make world politics after two liters.

08:12 This observation was the **basic** premise : to ingest a poison little by little. You can try it yourself, quit drinking alcohol for half a year, and then drink two glasses of wine, you'll almost fall over. But **not** because the body made the **anti**-poison, **but** because the body is trained, it has prepared the enzymes to quickly process and neutralize and excrete the alcohol. This doctrine of juices in this form is the **explicit basis of the entire western academic medicine,** including the **false belief in an immune system.** Why? They believed, that a disease-causing poison could develop, and if one **preemptively** took a poison, then the body would make an **anti**-poison, so one would obtain immunity. And whenever the disease-causing poison arrives, then I'm already invulnerable. That's why Rasputin and "Napoleon" (this can be measured in the hair) frequently took different kinds of toxins in small quantities in order to be immune against a poison attack. But then in the 19th century, when it was possible to **detect small** amounts of toxins, it was found that **in no illness,** toxins can be detected. **In non of them, until today.**

9:56 So the theory proved to be **wrong**. But the **entire** thinking was based on it, that means to give **mercury** compounds preemptively, as in **Goethe's** times, so whenever the disease-causing toxin comes about in form of an epidemic, the person is already invulnerable. This perception of **epidemics** already developed in the history

of ancient Greece. It was **claimed** that the illness **demon** would possess and defile a person. From this, the Greek word **miasma** is derived. Defilement, one is tarnished by the illness **demon** and one can **transmit** the disease to others. The disease demon infects me, reproduces within me, and can be passed on to others and disease-causing toxins would be produced. That's **why** anti-poisons are administered preemptively, just as **Goethe** describes it, and the **survivors applauded.** "And now from the withered old must hear **How men praise shameless murderers.**" As It were, **Goethe** revived **Paracelsus**, **in the** figure of Faust, but he is ashamed of having been celebrated by peasants before, as they call after him: "You saved our lives!" **but thousands perished.** He was ashamed of this and sat down with his **Atlantus Wagner** on this certain rock near the village and meditates and recounts what he did, and what his **father** had done.

11.56 It had been observed that **bacteria** produce **toxins**. This was investigated, and they were all very certain, that bacteria could **only** produce toxins in the **dead** body. That is because bacteria run their metabolism in an aerobic environment and produce substances in the intestines during digestion such as necessary vitamins. But when these bacteria are deprived of "oxygen", which **is the case in a dead body** after a couple of days, then a few of them can survive, they change their metabolism. Just as the yeast changes its metabolism under exclusion of

“oxygen” , to produce the **toxic** alcohol, in this way these bacteria produce their toxins, **but only under complete exclusion** of “oxygen”.

13:03 This was **well known,** bacteria **cannot be the cause** of disease. Professor **Henle** further **solidified** this knowledge, he phrased his postulates and said: **If you claim** that bacteria can be transmitted and then produce their poisons, **then you need to identify that bacterium in every case of a disease, which ,** you claim was caused by this bacterium. And that was **not** the case. Bacteria are **only in**sufficiently identifiable **in a test-tube,** it can be done **only** with a **few** of them. **Of all** bacteria, which we know based on their performance, **only about 2 %** are **cultivatable** and **multipliable**. And what is **defined** as a bacterium **in the** laboratory **is not the same as** the original bacterium outside. Why?

14:11 Because the **idea** of bacteria in the **lab** representing **one single type, is a laboratory artifact.** For, bacteria, exchange information among one another continuously and they change their form and function. This was recently **confirmed** in a large study: bacteria, as **individual** as they are in terms of their biochemistry, they are very **similar** in terms of their nucleic acid. They **adapt**. If we **extract** a bacterium and **cultivate** it in isolation, it **looses** its properties after some time and it

can't survive. Thus, I must produce a large quantity of them, freeze them and then I always work with those. But this already constitutes a massive **intervention** into nature, and **doesn't** represent the reality of bacteria exchanging their information amongst one another, and **thus the definition of** types that was imposed on them, was **not** scientifically justifiable. That was the **first** problem. For instance, they **didn't** manage to find the famous **tuberculosis** bacterium, the cultivation of which was successfully done by **Robert Koch:** It could **only** be found in about **half** the cases. That **remains** the **same** to the present day.

15:46 The second **Henle** Postulate states: this **isolated** pathogen must be observed, in case of a bacterium it must be multiplied, and it must be observed **whether or not it can** do what is assigned to it. In all these experiments they found, the bacteria **couldn't** produce toxins **in** the living organism, **only after** a few days in the **dead** body, **after** an animal or human **died**. That was also determined, throughout the **entire** scientific community **without** exception.

16:23 **Henle** formulated the **third** Postulate, which states: Then, the pathogen that was **isolated** and **multiplied**, must be injected into an organism and the **same** disease must develop. And this has **never** happened, **never** ever.

17:00 But how did it happen, that suddenly,**Robert Koch** was celebrated as the discoverer of the **transmissibility** of diseases? That is the question.

17:03 The question is easily answered. Robert Koch deserved**??** reputation for having managed to make photography adaptable to visual microscopy and to make photographs of **bacteria**. **Photography** itself had been **rediscovered??** in Europa in **1885**. This brought him much reputation deservedly. Photography was considered to be **sacred**, **no** one could imagine that a negative could be **retouched**, that **double** exposures could be used, that it could be **manipulated**. It was deemed as **inherently** scientific and objective. They simply made **claims** along with photography, and this acted in a very hypnotic way, much like television today, so people just **accepted** these claims.

18:00 He simply photographed bacteria that can be found everywhere. From this, **two** different concepts derived. Of course, these bacteria **don't** cause disease, but the **Third** Postulate (which states bacteria much **cause** the **same** disease) was **violated** by Koch, he introduced the scientific **fraud**, that plays the **central role** until today, in cases like AIDS, **vaccination**, influenza pandemic, and so on , including Tamiflu.

18:41 He said, the inoculation of the test animal with this bacteria **culture** leads to the development of a **similar** illness. Not , the **same**, but a **similar** illness. And this is one of the general acts of **fraud** of the entire infection **theory**: development of a **similar** illness. Read for yourself, that's homework number one, don't just believe me, go the library, **read what Robert Koch did.** Anthrax, just one **example**: he kills mice with corps toxins. This corps poison you can make at home: leave a potato salad standing outside in the summer for a weak, spoiled egg meal, bacteria spores are floating in the air everywhere, they settle, grow, reproduce, they consume the "oxygen". They transfer into the anaerobic state, mostly in the **centre** of the potato salad or the egg meal or in a dead body. And then, toxins are produced. The toxins themselves they can **kill**, **if** this is fed to a person little by little, and the foulness is covered up with strong spices **or taste enhancers.** In this way, a person can be chronically poisoned, or be caused to suffer severe diarrhea and cramps.

20:10 **Koch** produced these toxins in a meat broth, as you can replicate at home, he **injects** them into the vein of a **mouse**, the mouse dies, the milt is swollen, he extracts the milt of the mouse, and transplants it under the skin of a **frog**. The frog convulses and dies and this is called **skin anthrax**. **Robert Koch, scientific fraud.**

20:42 Now you can imagine which animal experiments were made **to claim lung anthrax.** The milt of the killed mouse was implanted into the **lungs** of the frog. That is what is done **until** the **present** day, that is what is done in the **influenza pandemic:** Animals are being killed with incisions of the **trachea**, liquids are inserted continuously, the animals die, and then it is **claimed**, it was the **virus**.

((You can study this on our influenza **virus** information flyer, which is attached to this file. On this you will find the literature on how they operate concerning influenza. **No control group** animals, if they were to inject ordinary liquids into the animals, the exact same were to happen.))

21:32 The second thing that was derived from **Germany** and **Robert Koch,** was this: Robert Koch relied on **new colorants** to be able to **dye** bacteria. And naturally, he received these dyes **from** the colorant industry. Then, **all other** medical researcher took the **same** colorants, took healthy tissue, they **acidified** the tissue and discovered they had the **same** coloring reaction and the **exact same** bacteria can be seen and photographed, **just as** Robert Koch did.

22:12 But then they **also** discovered, these **dyes killed** bacteria by making holes in them, they inhibit the DNA**??** of the bacteria, these can **no longer** reproduce, the bacteria **die**. **From this, antibiotics were derived,**

from the colorants. **BASF, BAYER, IG** Farben, Hoechst, Merk and so on. The **pharma- industry was derived from colorant manufacturers based on the infection hypothesis.**

23:24 But **why** did the German **government** employ **Robert Koch**? He already had to **flee from Berlin** before, because **he had killed thousands, with his magic drug tuberculin against tuberculosis.** This drug's ingredients were kept **secret** , against the law. He fled, **Otto von Bismarck** called him back. , he desperately needed a **pretext** against the British who had seized the Suez canal and thus had **significant** military and political advantages, for they didn't have to sail around Africa, but they came through the Suez canal **from India** with their troops and goods, such as spices, serving as anti-oxidants, and the like and they sailed through the calm Mediterran sea. The German tried to deprive the English **form** this advantage with the **allegation** , they were bringing home, **anthrax, smallpox, the black pledge from India.** Thus, **quarantine** was demanded, they **weren't** allowed to dock at any Mediterranean port and at Gibraltar they were shot at. Therefore, **Robert Koch,** who was on the run, was called back and was offered 100,000 Reichsmark **in order to create the argument that** the English would bring in **black plague, small pox and anthrax,** the latter we already discussed. We noted, that this colorant business lead to the **emergence** of antibiotics, later to the

chemotherapy and the **weaponized** gasses, including the pharma- industry with its entire capital, **with more revenue then all** military budgets **globally** combined.

25:18 **Robert Koch** committed **scientific fraud by not upholding the first** postulate. He could cultivate some bacteria, which he **didn't** find in **every** case of a disease. This is **still** done in the **same** way today. He **could never reproduce the disease as in the third** postulate, and **neither** could he again **isolate** the **same** pathogen from these organisms. That **is the date when the brutal animal experiments were introduced.**

26:02 **How did the idea of a virus come to life?** Koch's French counterpart was **Luis Pasteur,**scientific **fraudster** employed by the **French**, as the French were **at war with Germany** in 1872. The **dead were later declared as victims of a small pox epidemic.** The Germans **claimed** it came **from** France, the French said it came **from** Germany. **Pasteur**, knew from **Bechamp** and other scientists, **what bacteria could do and what they couldn't do,** he **first denied** the **new** knowledge in order to play along with the **church**, he **claimed** he had **proven** the **primordial** creation, **only** to later take a **reverse** stand, once he was **employed** by the state, he said it was all **wrong**, bacteria are in a continuum, there are spores, they **cannot** be created in the primordial soup. **Pasteur**

sold these insights **as his own, but he knew** what bacteria can and what they cannot do, and has earned some merits for this.

27:16 But the same **Pasteur**, who **knew**, that bacteria **cannot** cause diseases, applied a **trick**. In order to maintain the model of the doctrine of juices and disease, which the **entire** western medicine is based on, a disease-causing toxin **had to be** postulated. Especially since this **concept** of pandemics had been used **many** times to suppress upheavals, to control starvation situations and so on. It all started, with the early **Vatican** creating fear of diseases, by claiming the disease is coming **from** the disease **demon**, just as in ancient Greece. Thus, in order to establish **total control**, the early Vatican claimed that illness was a punishment by God.

28:26 This concept was **interrupted** for a few years by the Stauffers, when **emperor** Otto, at the **re-fou**ndation of the **Holy Roman Empire** on Jan 1st, 1000 AD employed the French Humanist **Gerbert de Aureac** for the purpose of establishing a medical system, which was obviously **not** present before. For, the **military** faction, the western Roman wing, had separated from Rome, that is well known. They had **only** military knowledge, but **no** technical knowledge, they were separated from the universities and culture. The early gothic was able to

build small windows only, no tall buildings, the building plans were useless, whenever the knowledge of craftsmen and engineers, which always must go together, didn't converge. This is visible in early gothic architecture.

29:33 So, they recruited Gerbert de Aureac, alias **Pope Silvester.** He brought in the Arabs in each garrison from which the **monasteries** were derived from, with the aim to obtain the ancient knowledge of the Chinese via the Arabs, who themselves had further developed medicine. For, in **China** the concept of contagion **didn't** exist, there is the concept of too much and too little **energy**, there is the influence, **latin** influenza, by the decrease and increase of **light** and **warmth** in **spring** and **autumn**, but the idea of contagion plays **no** role. Contagion is **not** part of arjuvedic medicine. The concept of contagion is typically **war**- oriented, **European**.

30:12 The idea of disease being something vile , was already retracted from by **Galenus**, the great physician of Marcus Aurelius. He refrained from this concept and stated that they had recognized: it **wasn't** sin that makes people sick, but rather that diseas may **cause** sins. And today, if we think about the insights of German New Medicine by Dr. Hamer, this is becoming very up to date. We find **psychoses**, that can be visualized in CT scans, as a result of diverse constellations, that is, activities in the brain in different locations and sudden alterations from

mania to depression and other properties. Gerbert de Aureac again pursued this (oriental) approach and the foundation of the empire was humane. However, this was quickly **changed** to the contrary, when Pope Silvester **died** a few years later. **After** this, health **tribunals** were installed throughout the entire Holy Roman Empire of German Nations.

31:26 The Vatican **didn't** manage to confiscate all the documents in all archives from this time, especially in the later **protestant** regions. From these city chronicles and book we learn that there were health **tribunals** in the entire Holy Roman Empire, **headed** by a priest, accompanied by community or city councilors, and **they decided** who was a **sacred sick** person, or who was an **evil sick** person, who was punished by god and therefore needed to be **expelled**. The **German** word for **leprosy** is **Aussatz**, which means **to cast away.** This leprosy / expulsion- concept is **identical** in **all** regions in which the data was preserved throughout the Holy Roman Empire in the beginning of the **11th** century. The definition included natural illnesses such as **hair loss, acne** zits, swellings and so on, but it also included **trickier** diagnoses such as the claim someone had a **nightmare**. He might have called out in his sleep or the like, and even more wicked (this could be called the **first** AIDS test in medieval times): **goose bumps** as a reaction to a draft. That was a criterium to be expelled, the person was **tattooed**

(marked), received the **last** oiling, and was forced to leave **all inhabited** territories, and was **forbidden** to come near a settlement the by threat of **death** penalty.

30:58 That was the leprosy concept (i.e. Aussatz, expulsion) beginning with the 11th century. Then, after the onset of the Small Ice Age in **1308**, when great pressure of **migration from** the north arrived, as the northern **apple** and **wheat** plantations became **less** productive due to **severe cold**, large tensions, hunger catastrophes and so on, arose in the **new** Holy Roman Empire of German Nations. Especially after the strong **earthquake** with epicenter in Friuli of **1348**, which **devastated many** Mediterranean cities. This was interpreted by the **orthodox** as a proof of the antichrist, as law and order **collapsed** because the **central** hub of **global** trade, **Venice**, as well as **all** trade lines and **currencies**, **also** collapsed. At this point, this **exact** concept with the **exact** same disease definition was adapted by the priests **and** city officials, to **declare entire** groups of the population as punished by god and they were **claimed to have the black plague. Entire city quarters were quarantined, put under lock down, starved to death, slaughtered and poisoned, just as Goethe described it.**

34:34 They simply **renamed Leprosy** into **black plague.** Later, as the Vatican's power of definition was reduced by national revolutions, French Revolution, American Revolution, the same concept was **renamed** into **small pox,** but the **same** principle remained. **Today, it is carried on in unacceptable disease definitions such as** AIDS. At any rate, the public was terrified to no ends, whenever epidemics were declared, for this meant they could be put into **quarantine**, they could be **killed**, they could be **forced** to take medications, **just as** Goethe described. Meanwhile, thousands **died** because there was **no** food, there were social upheavals. And the **survivors applauded.** This medical system was **always** immediately **repressive** in times of crisis and in its history it always regarded disease as something vile, evil: the illness **demon**, that **takes hold of** someone and grows and rages like a cancer, &**above all,** can even be **spread** and **transmitted** to others like an **evil spell,** the disease **demon**. This **fear** was extremely ingrained in society, and the medical system, **from which** the pharma industry as the **most** powerful entity on this planet, arose, will **not** give up this power on their own account. For this, we need to become **active** citizens. For this, I will provide you with more information. The idea of a **virus** was realized, and **from** this idea the field of **gene** technology was derived.

36:32 To pick up with **Pasteur** again: Pasteur knew that **bacteria** could **not** cause diseases, period. Enough studies and experiments were conducted and published in Germany and elsewhere, among other by **Max von Pettenkofer,** who demonstrated what **cholera** was and how cholera was **easily** prevented. **Pasteur** worked on contract to find an **argument** , to not let the English through the Mediterranean Sea, he came up with the **idea** to **claim** there was a **new** pathogen, and this one would make its disease-toxins also in the living human body and this he called: poison! Latin: **virus**.

37:22 That was the idea. He said it is a **thousand times smaller then bacteria,** we use such dense filters where bacteria **can't** pass through. He presses the liquid, the poison **from** a **dead** animal, through the filter, he injects the liquid **into** the **brain** of a **dog** that was tied onto a pole vertically. He used a third of the volume of the dogs brain, the liquid comes out the over side, the dog convulses, barks, foams from the mouth and dies. That was called **rabies**, that's what **Pasteur** did.

38:20 Pasteur **also claimed** to have the **antidote** to his **virus**, to push the **vaccine** concept. This vaccination agenda was propagated **primarily** in France, for the **Germans** had their antibiotics and chemotherapy. Pasteur committed **fraud in all** his undertakings. But he was humane enough to document his deceits in diaries parallel

to his primary lab books. He decreed that these records must never be publicized. His family naturally obtained great wealth. But the last male ancestor of Pasteur didn't obey to that decree and leaked the records to the Princeton university and in 1993 Professor Gerard Geisson published an analysis in the English language that revealed that **Pasteur** had committed **massive fraud in all** his studies. For instance, vaccinated animals, **if** they survived, had not been poisoned, the control group animals that died **without** vaccines were **poisoned massively** and so on. **That was Pasteur.**

39:30 **Pasteur** is the **inventor** of the **idea** of a **smaller** pathogen that can**not** be seen in the optical microscope, **but** that always makes its poison, the disease- causing poison. This supported the standard model of illness which was used for centuries, a model that is **based on the premise of war, not** on the premise of symbiosis, as is the real workings of Nature. In order to solidify this model and to have **political** leverage against England, **Pasteur** postulates the idea of a **virus**. But Pasteur didn't anticipate that there would be a **microscope** in the future, an **electron** microscope, which has a much higher magnification as the optical microscope, that would allow to see small structures **not** visible before. And with this **electron** microscope, available to science after WW II, it was possible to **visualize??** structures one thousands of the size of a bacterium. They observed spores, that were

still capable of **staying alive.** It was recognized that bacteria generate spores, **when** they die **slowly**. **If** they die **rapidly**, when they are for example **heated** or dried out, then they produce **even** smaller particles that can't live by themselves, but they consist of proteins and bear a nucleic acid in the center, and they will provide other bacteria, the ones that survived**??**, with nutrients, so the latter can overcome the crisis situation.

41:22 This was observed in the cases of bacteria, in other very simple organisms, in fungi, in amoeba. In my own research, I first found it in a very simple algae from the ocean. But it was **never** observed in a human or animal or plant. You can verify this with little effort.

42:40 As a **first** step, you can check the **virus** question by asking: How are **viruses** detected nowadays? If a virologist claims he **isolated** an influenza virus, **then** he refers to the chicken egg and the chicken embryo, as we can see in the media for the planning for the current H1N1 influenza **pandemic**. They work with chicken eggs, they **kill** chicken embryos, **that's the modern form of animal tests.** This method goes back to **Robert Koch. If** the embryo **dies**, they say it was a **virus** and that they had **isolated** the virus. They took something from a **diseased** animal or a human, they **inject** it into the egg and then, depending on **how** the embryo dies, on **what** location it is becoming spotted first, they will **claim** it was

this type of virus **or** that one. **That is proclaimed to be isolation,** when chicken embryos are killed.

42:44 Needless to say, there is **no** control group: if you inject a **sterile** solution of the **same** amount, the chicken embryo **dies as well.** You can also verify it by taking a look at the **photos** that claim to depict **viruses** and you will find they are **identical** with images from completely **normal** cells, that is with **electron** micrographs from normal "cells". "Here", we see a section from the centre of a cell, which is very productive, the so-called **Golgi** apparatus, that produces various substances, and these are separated in small vesicles, they are all of different sizes, but they have **no** nucleic acid within them.

43:32 A larger **fat** particle is called a **small pox virus,** here we see the bacteria within the "cells", the mitochondria, which process the oxygen, here we see two small ones. These particles are mitochondria, a muscle "cell" has 1000, a liver "cell" has 2000 mitochondria, and these were extracted from the "cell" in a thin cross section, embedded in artificial resin and cut in a very thin slice with a diamond blade. When they are protruded with an "electron" beam, we see the cap of such a mitochondrion. And **such particles are then sold as virus without** ever having **isolated** them, **without** having shown them in an **isolated** condition, in order to

demonstrate that these look identical to what is shown in the photo of the “cell”.

44:32 “Here”, for instance, the photo that circulated as the **photo** of HIV, published by **Montagnier**. “Here”, we see how particles are excreted or absorbed from the “cell” into or out of the “cell” cavity. In biology, we call this endocytosis or exocytosis. Whenever something goes into or out of the “cell” liquid. This has nothing to do with particles that have a **stable** structure, that carry nucleic acid within them, that can leave the organism, such as the **viruses** or bacteria, that are called **phages**, or with the things I discovered in the ocean, they are harmless. These particles here are normal components of “cells”. In the case of the funny photos that are presented as influenza **virus**: these are **simply** mixtures of **fats** and **proteins**. If these are shaken in an ultrasound bath and then visualized (they decompose quickly) they don’t have an nucleic acid and they are of various sizes. That’s how you can tell fraud.

45:45 Further, they **don’t** even claim that they observed this **within** the cells or that the particles were **isolated**. What is striking in the current swine flue **pandemic**: they are trying to present more pictures of **particles** that are more or less **equally** shaped. You can research this by demanding a scientific publication, in which it is proven and documented that the **virus exists**, it was seen in the

organism, it was **isolated** from the organism, **purified** from all foreign components. Just as on world savings day [or when you fill out your Tax form], your Euros must be isolated, buttons and chewing-gums are not accepted. Then the **isolated** particles must be **analyzed** biochemically. In the **first** step, a scientific publication can be recognized in the title of the scientific **journal** and of course the two dates, **first** the date of submission of the paper to the editor and **second** the date when it was checked and accepted by three work group, that were not previously known to the authors**??**… translated by Sacha Dobler
https://abruptearthchanges.com/2017/11/17/dr-stefan-lanka-the-history-of-the-infection-theory

There's **renewed controversy** surrounding influenza vaccines today, with some studies showing people immunised against the seasonal flu may have been at **greater** risk during the swine flu outbreak. There are calls for a review of Australia's policy on vaccines, but the Chief Health Officer says the evidence is patchy.
https://www.abc.net.au/radio/programs/worldtoday/new-controversy-surrounding-flu-vaccination/1968054

Stress Hormones and Immune Function
Jeanette I Webster Marketon 1 , Ronald Glaser

Over the past 20 years we have demonstrated both in animal models **and** in human studies that stress increases neuroendocrine hormones, particularly glucocorticoids and catecholamines but to some extent also prolactin, growth hormone and nerve growth factor. We have also shown that stress, through the action of these stress hormones, has detrimental effects on immune function, including reduced NK cell activity, lymphocyte populations, lymphocyte proliferation, antibody production and reactivation of latent viral infections. Such effects on the immune system have severe consequences on health which include, but are not limited to, delayed wound healing, impaired responses to vaccination and development and progression of cancer. These data provide scientific evidence of the effects of stress on immune function and implications for health.
https://pubmed.ncbi.nlm.nih.gov/18279846

Vaccine derived virus interference was significantly associated with coronavirus and human metapneumovirus
https://www.ncbi.nlm.nih.gov/pubmed/31607599

https://www.psitalent.de/Englisch/Spanish%20Flu.htm
Spanish Flu 1918 ,caused by vaccination.

Dr. rer. nat. Stefan Lanka demanded other scientists to demonstrate a proof of vicious viruses. Nobody could. Stefan Lanka's work is very convincing.

Interview with virologist Dr. Stefan Lanka
Interview by Christopher Ray - October 27, 2007

Q…Dr Lanka, are we threatened in Germany by the Bird Flu?

Only **indirectly**. Next year, in Germany there will be **much fewer babies**. If one follows the media, the storks will all have been snatched away by the bird flu. We are supposed to believe this.

Q…Do you mean that seriously?

Just as seriously as there being any danger for us from the alleged bird flu virus H5N1. **The danger or the disaster lies somewhere else entirely.**

Q…Where, in your opinion, does the danger or the disaster lie?

We have allowed ourselves to loose the habit of using our reason. That is the actual danger of the disaster. The politicians and the media are so shameless as to make us believe that that migratory birds in Asia have been

infected with an extremely dangerous, deadly virus. These mortally diseased birds fly then for several weeks. They fly thousands of kilometres, and then infect, in Romania, in Turkey, Greece and elsewhere hens, geese or other poultry, with which they have had no contact, and which within a very short period get diseased and die. But the migratory birds do not get diseased and do not die, but keep on flying, for several weeks, thousands of kilometres. Anyone who believes this will believe too that babies come from Storks. Certainly the majority of people in Germany believe in a danger from Bird Flu.

Q…Is there then no bird flu at all?

Since the end of the 19th century, diseases of poultry have been observed in mass animal farming: Blue colouring of the crest, decrease in egglaying performance, lacklustre plumage. And sometimes these birds die too. These diseases were **previously** called Bird Pest. In current poultry farming, in particular when hens are being raised in cages, many birds die each day as a result of this alien manner of animal farming. Later, these consequences of the mass animal farming were **no longer** called Bird Pest, but Bird Blu. We are witnessing that a contagious virus is being **alleged** , as the **cause** of this , for decades , in order to deflect from the actual causes.

Q…Then those 100 million hens which appear to have

died from bird flu in reality have **died from stress and/or from nourishment deficiency and poisoning?**

No! If **one** hen lays fewer eggs or gets a blue crest and that hen is tested H5N1-positive too, **then all the other hens are gassed.** That is how the apparently 100 million killed hens from H5N1 was achieved. If you look at this more closely, then you see behind it **a decades-old strategy:** In the West, the big enterprises are **restructuring** themselves with this, because those animals which have **died** from the ‚contagious' disease are **being compensated for at the expense of the general public, at the highest market price, while in Asia and** everywhere where poultry are being farmed successfully, the poultry market there , is being destroyed maliciously and deliberately under the leadership of the UN organization FAO (Food and Agricultural Organisation). All **big** Western poultry breeders therefore, are keeping their mouths shut and by means of their veterinarians are seeing to it that, if the market price for poultry **sinks**, they get an infectious disease **diagnosed**, so that they can get their birds sold **for a higher profit than** would be possible with normal farming, disposed of **all at once at the** Government guaranteed maximum price. The common denominator: It is a modern subvention fraud along with paralyzing scaremongering, which as a secondary effect **guarantees that nobody asks for evidence.**

Q…Of what did those **61** persons **die** who were proven to have H5N1?

There are **only very little very few publicly accessible reports available,** describing what the symptoms were and **how they were then treated.** These cases are explicit: Persons with the symptoms of a **cold**, who then had the bad luck to fall **into the hands of** H5N1 **hunters**, were **killed with insane quantities of chemotherapy supposed to restrain the phantom virus.** Isolated in plastic tents, surrounded by madmen in space suits, they died, in panicky **fear** , from **multiple organ failures.**

Q…Does this bird flu virus then not exist at all?

Structures have **never** been seen or proven in humans, in the blood **or in other** bodily fluids, in an animal or in a plant, which one could characterize as bird flu viruses or flu viruses or any other allegedly pathogenic virus. The **causes** of the diseases which are alleged to be caused by a virus, also those in animals, which can arise quickly, one after the other or simultaneously with several individuals, are **well known** for a long time. Even more: There is simply **no place in Biology really for viruses as instigators of disease.** There is only a place for **fantasies** such as disease-inducing viruses, if I ignore the discoveries of the New Medicine by Dr Hamer, according to which **shock** experiences are the **cause** of **many**

diseases and ignore the discoveries of chemistry about the effects of poisonings and deficiencies and ignore the findings of physics about the effects of radiation.

Q…Why then are pathogenic viruses still being maintained to exist?

The Orthodox medical doctors **need** the paralyzing, stupifying and destructive **fear** of disease inducing phantom viruses **as a central basis for their existence:** Firstly, **in order to harm many people** with vaccinations, **in order to build up** for themselves a clientele of chronically ill and ailing subjects who will allow anything to be done to them. Secondly, **in order not to have to admit** that they are failing totally in their treatment of chronic illnesses and have **killed and are killing more people than all the wars up to now** so far have achieved. Every orthodox medicine practitioner **is conscious of this,** but only very few dare to speak about it. Therefore it's no wonder either that among professional groups, it is that of the orthodox medicine practitioners that has **the highest suicide rate,** far surpassing other professional groups. Thirdly, the Orthodox medical doctors need the paralyzing and stupifying fear of diabolical viruses, **in order to conceal** the early middle ages **origin** of the ‚white coats' **as a** transparent Killing and Torture instrument of the inquisition. Orthodox medicine was and is the **most important pillar of** support

of all Dictatorships and Governments which do not want to submit to written law, to constitutions, to human rights, that is, to the democratically legitimized social contract. This explains too why Orthodox medicine really can and may do anything, and in this is **subjected to , no control** whatsoever. If we do not overcome this, **we will all be destroyed by Orthodox medicine.**

Q…Are you not exaggerating a little here?

Unfortunately, no! Anyone who opens his eyes will see it that way. **Ivan Illich** warned about this already in 1975 in his analysis The Expropriation of Health. This book still exists today, under the title: Medical Nemesis. **Goethe** described the state of orthodox medicine very aptly in Faust I and has the physician Dr Faustus admit:

*Here was the medicine, **the patients died and nobody asked who recovered,** thus we have ravaged with infernal Latwerges, in these valleys, these mountains, much worse than the pest, I myself have given the poison to thousands, they withered away, I must witness that **the unabashed murderers are praised.***

Goethe calls Orthodox medical doctors, Latwerge, that is they give **poisonous** substances, unabashed murderers, who are still today being praised. Here I can and also must refer to our publications, because we, the first to do

so, have put the central revealing question to modern medicine and have documented and commented on the resulting admissions. At http://www.klein-klein-verlag.de/ you will find all the relevant information on this.

Q…Why have just you hit on this thousand year old fraud?

I have studied molecular biology. In the course of my studies I demonstrated the existence of the first virus in the sea, in a sea algae. This proof was first published in a scientific publication in 1990, in accordance with the standard of the natural sciences. The virus whose existence I demonstrated **reproduces** itself in the algae, can leave it and reproduce itself again in other algae of this kind, without having any negative effects, and this virus has no connection whatsoever with any disease. For instance in one litre of sea water, there are over 100 million viruses of various kinds very different to each other. Fortunately, the health authorities and the doctors have not become aware of this, **otherwise there would have long ago been a law allowing sea bathing only in total body condoms.** One has **never** seen Biological structures however, which would do anything **negative**. The basis of biological life is togetherness, is symbiosis, and in this there is no place for war and destruction. War and destruction in biological life can only be alleged by a

sick and criminal brain. In the course of my studies, **I and others have not been able to find anywhere proof of the existence of disease-inducing viruses.** Later we have lectured on this publicly and have called on people not to believe us either but to verify themselves whether there are disease-inducing viruses. Klein-klein action emerged from this, and has for 5 years been asking **the health authorities for proofs and which finally has gotten admission of and certainty of the fact that there is no proof of disease-inducing viruses and no proof of any benefit from vaccination.** We founded the klein-klein-verlag (publishing house) three years ago, in order to enable us to genuinely make these results publicly known.

Q…What viruses are there then at all, and what do they do?

Structures which one can characterize **as** viruses were detected in many species of bacteria and in simple life forms, similar to bacteria. They are the constantly independent elements of living-together of different cells in a common cell type. This is called a symbiosis, an endosymbiosis, which has ensued in the course of the process of different cell types and structures combining, **out of which the present cell type has arisen** that Humans, Animals and Plants consist of. The viruses are component parts of cells, as the bacteria in all our cells

which assist the transfer of oxygen, the mitochondria, or the bacteria in all plants which produce oxygen, the chloroplasts.. Very important: Viruses are component parts of very simple organisms, as an example of Fibre Algae, a particular type of a uni-cellular Chlorella Algae and of very many bacteria. These viral components are called Phagen. However **such structures which one might call viruses have never been seen in more complex organisms especially in humans, or in animals or plants.** In contrast to the Bacteria in our cells, the Mitochondria, or the Bacteria in every Plant, the Chloroplasts, which cannot leave the common cell, since they are instructed by the metabolism of the common cell, Viruses can **leave** the cell, since they are not carrying out any important survival tasks within the cell. Viruses, thus, are component parts of the cell which have given their entire metabolism over to the common cell and therefore can leave the cell. Outside the common cell, they help other cells, in that they are transferring building and energy substances. One has **never** observed anything else. The actual scientifically proven viruses perform in the highly complex interactions of the cells a helpful, a supportive and in no way a destructive function. Actually **in diseases, neither in the diseased organism nor in a body fluid, one has never seen or isolated a structure which one could characterize as a virus.** The allegation of the existence of any disease inducing virus is a transparent **fraud**,deadly lie with dramatic consequences.

Q…Are you not alleging with this that the dangerous AIDS virus is also only **virtual**?

It is not only I that alleges that the so-called AIDS virus HIV has **never** been scientifically proven, but that **it is only said to be proven only on the basis of a consensus.**

The Federal Minister for Health in Germany, Ulla Schmidt, wrote on 05.01.2004 to the Member of Parliament Rudolf Kraus: Of course the Human Immunodeficiency Virus is said - according to international scientific **consensus** - to be scientifically proven. Today, the Federal Health Authorities (Germany) **no longer** allege that any so-called disease-inducing virus has been **directly** proved, after citizens for years have time and again **questioned** the Federal Health Authorities for **scientific proof** of the existence of the allegedly disease-inducing viruses. In an ongoing process of petitioning to the German parliament, the Federal Health Ministry shifted all responsibility onto the Federal Research Ministry. The Federal Health Ministry is taking the absurd standpoint that the constitutionally guaranteed freedom of science prohibits the state from verifying scientific allegations.

But that is absurd. That would mean that the State is surrendering us , helpless and protectionless, **into the**

hands of an uncontrolled science which does do and can do what it wants.

Q…Are you really of the opinion that the State is surrendering us in such a manner?

I do not have an opinion here. I can only see and state the facts. We are experiencing with the current Bird Flu panic that **the State, in Germany, contrary to its best knowledge, is surrendering the population into the hands of some people who are masquerading as Scientists.** An **enforced** chemotherapy is being planned, and next spring the entire German population is to be **forcibly** vaccinated twice against the alleged Bird Flu **Phantom**. But **neither has ever a bird flu virus been proven, nor was any virus proven, that would have any connecion with a disease.** Such **viruses do not exist.** Precisely in the same manner as the Minister admitted concerning the alleged AIDS virus, **they are alleged to be proven and therefore existing because of an international scientific consensus.**

Q…But the bird flu virus H5N1, dangerous to humans, was quite recently very precisely proven in an English laboratory!

If ever a virus coming from a specific body or a body fluid, for instance from birds, has **been proven,** then any

average scientist can verify, in any average laboratory, within a day, whether this virus is present in , for instance a dead animal. This has however **never** occurred, and on the contrary, **indirect test methods which tell absolutely nothing** , are being used. For instance, it is alleged that there are **antibodies** which would combine with the body of the **alleged** virus and **only with it,** and with the evidence of a successful combination between body and antibody, the existence of the alleged virus would be proven. **In reality, those alleged antibodies are soluble blood proteins,** which **play a central role in** the sealing of cells which are growing and dividing and in the healing of wounds. These blood proteins, also called globulins, will combine arbitrarily with other proteins in a **test-tube** containing appropriate concentrations of acids and bases, minerals and solvents. Thus you can make any sample taken from an animal or a person test **arbitrarily** positive or negative. It is pure, and this must be quite clearly said, **criminal arbitrariness.**

Also if it is **alleged** that, by means of a biochemical multiplication technique called Polymerase Chain Reaction (**PCR**), the so-called gene substance of the virus can be multiplied and thus was proven. This is a **Fraud**, since **firstly**, **there exists nowhere a gene substance of a disease inducing virus, to which one might compare the artificially multiplied particles of gene substance, and secondly, only such parts of gene substances get**

multiplied which already existed earlier in those fluids which are used for indirectly demonstrating the existence of the alleged virus. And it is quite simple: A thousand pieces of **indirect** proofs, corn circles for instance, do not make a UFO either. You do not even have to know English, so as to read those publications to which the virus **fraudsters** are referring, in order to see for yourself , a virus does not appear anywhere in them: If you ask those scientists **for proof of the existence of the purported viruses,** for instance that of H5N1, you will get only **evasions** in return and **never a concrete answer.** On TV we have heard time and again that the investigations were carried out **in an** English laboratory. The **name** of that English laboratory the public has not gotten to know. It is the reference laboratory of the EU for bird flu, in Weybridge. I have asked the scientists several times for the pieces of **proof** of the existence of the H5N1 virus. They have replied to me **only once, and after that never again, and wrote that they did not** understand my question. I also have written several times to the World Health Organization and in particular to the bird flu pandemic co-ordinator, the German **Klaus Stöhr,** and asked for proof of the existence of the Bird Flu virus. **Neither the WHO nor Klaus Stöhr has reacted to this.**

Q…What, however does this H5N1 **mean** that the whole world is afraid of ?

The H in H5N1 stands for Haemagglutinin. the N Stands for Neuraminidase. The **Pseudo-Virologists** allege that the protein of the type Haemagglutinin and the Protein of the type of Enzyme Neuraminidase **are found in the coat of the Flu virus.** **Not only are** the proteins which are lumped in with red blood corpuscles called Haemagglutinin in Biochemistry **but the many different substances are also called Haemagglutinin.** The **pseudo-virologists** have agreed among themselves that there are supposed to be **15 different types** of Protein with the characterists of a Haemagglutinin in the coat of the Flu-**virus**. The 5 is **for type 5** of an alleged Protein which somehow has **only** been **indirectly** proven.

Now in order **to detect** a Flu Virus, red blood-corpuscles are mixed in the laboratory, **with samples in which the alleged** virus is supposed to be. **If** the red blood-corpuscles lump together, **then it is alleged that** a haemagglutinin **in** an influenza virus **must be the cause** of it, **without a virus having been isolated from a sample or from such a mixture let alone ever seen in it. From the manner** of the lumping together, precisely like the Seers in Asterix and Obelix did it,they **deduce**, **which** type of haemagglutinin it is supposed to be.

These scientists have a multitude of test procedures, which by their construction guarantee that , precisely that type of haemagglutinin is shown which the testing

scientist already have **assumed** in advance. **It is exactly the same with** the enzyme Neuraminidase, which is **alleged** as a component part of the coat of influenza viruses. **There are nine different types , alleged to exist here by the Pseudo Virologists.**

In **reality** the Neuraminidase is an enzyme which by the separating from parts of an Amino Sugar called Neuramin acid regulates that surface tension which is decisive for the functioning of the respective metabolism.

Analogously to the viral haemagglutinin , there are **a host of** Test Procedures that one can **purchase** which demonstrate exactly the result, that is the type of neuraminidase, which the **clairvoyant** virologist already had **assumed** in advance. Therefore it is also **no** wonder that that turkey cock, which **ostensibly died of** H5N1, belonging to the 73 years old farmer Dimitris Kominaris on the East Aegean island of Inousses, **has vanished without a trace, that at the reference laboratory in question demonstrably no sample from Greece has arrived, and that the clairvoyant media however have reported that a first sample has confirmed the suspicion.** In order to detect H5N1 **no** sample is necessary either, since, as with all purported contagious diseases, it is a question of a **planned action, intended for political reasons to induce fear.**

Q…In the media, **photos** of bird flu viruses and influenza viruses are constantly shown. Some of these photos show round formations. Are these not viruses?

No! Firstly, those round formations which are supposed to be a Flu-virus, are recognisable for **every** molecular-biologist as **artificially** produced particles of fats and proteins. The layman can verify this by asking for a scientific publication **in which these pictures are reproduced and described and their characterisation documented. Such a publication does not exist.** Secondly, the pictures which are supposed to show Bird Flu Viruses, are for every Biologist clearly recongnizable as **quite normal component parts of cells, or even show complete cells which happen to be in** the process of exporting or importing Cells and component parts of metabolism. Again, the layman can quite simply verify, by asking for those publications which those photos come **from.** He will **never** receive such publications. The **scaremongerers'** Guild doesn't want to reveal its means of trade: **Fraud** with laboratory experiments and animal experiments. If you ask the picture and news agencies **where they are getting their photos from,** then they will refer you to the American contagious-disease authority the CDC (Centre for Disease Control) **controlled by the Pentagon. The only photo of the alleged H5N1 comes from this CDC.** This photo shows the length-and simultaneously the cross section cut , of tubular structures

in Cells which have been **killed** in a test-tube.

Q.. It has been demonstrated however that H5N1 kills hen embryos and can be cultivated in eggs. What is the catch?

These experiments have already been used over a 100 years ago, **in order to prove the existence of quite different viruses, for instance of the alleged smallpox virus.** In this way, **extracts** are injected through the eggshell into the embryo. Depending on **how much** is injected and **where** in the embryo , the seemingly virus-infected extract is injected, the embryo dies faster or more slowly. It would die from such injections in precisely the same manner too **if** the extracts were sterilized in advance. This killing then is presented by those **virologists**, firstly, **as direct proof of** the existence of the respective virus, secondly as proof of the possibility of **multiplying** the virus, and thirdly and simultaneously as proof of the **isolation** of the virus. **Various vaccines are produced from hen embryos killed in this way, millions** of which are dying silently each year at the vaccine manufacturers. There are, besides hen embryos, also cells are being killed in **test-tubes in order to present the dying of these cells as proof of the existence, the multiplicaton and the isolation of a disease inducing virus.**
Nowhere however is a virus isolated from this, photographed in an electron microscope and its

component parts described in processes which are called electrophoresis.

Q…But then what kills the animals in the animal experiments, if it is not the H5N1?

One only has to examine at the publications in which these animal experiments are described. Hens are slowly **suffocated** to death within three days by means of administering **a fluid to them through** the windpipe tube. In small Java monkeys, 30 days **before** the **alleged** infection, temperature **transmitters** are being placed in the abdomen, 5 days before the **alleged** infection they are fixated in a **negative** pressure chamber. And **at the** point of the so-called infection, an amount of liquid corresponding to six schnapps glasses for humans is pressed through the tube in the windpipe of these young animals. Parts of the **same extract from dying,** that is putrefying, cells are injected into both **eyes** and into the **tonsils** of the animals. In many cases, suffocation attacks are caused in the animals **by means of rinsing the bronchial tubes, etc.**
The **resulting damage and destruction is then presented as the result of** H5N1. I have informed the former Minister for Consumer Protection, Künast, and the present minister, Trittin, who are presenting themselves as protectors of animals, about this, via their personal Spokesmen. There was no reaction.

Q…But the virus of the **Spanish** flu has been reconstructed genetically and also has been demonstrated to be a bird flu virus!

What, so to speak, was reconstructed genetically is nothing else than a model of the genetic substance of a Flu virus. A Flu virus has **never** been isolated. A genetic substance of a Flu virus has **never** been isolated either. All that has been done is multiplying gene substance **by means of the** biochemical multiplication method Polymerase Chain Reaction. **With this** method it is also possible to multiply **arbitrarily new, never previously existing, short** pieces of gene substance. Thus it's **possible** with this technique **also to manipulate the genetic fingerprint, that is, to test someone as identical to or different from a sample that has been found.** The genetic fingerprint will provide a certain **probability** of a match, **only if a lot of the gene substance that can be compared is found.**

Dr Jeffery Taubenberger, who created the allegation of a reconstruction of the **1918** pandemic virus originates, **works for the US-American army** and has worked for more than 10 years on producing, on the basis of samples from different human corpses, short pieces of gene substance **by means of** the biochemical multiplication technique PCR. Out of the host of produced pieces he has

selected those which came closest to the **model** of the genetic substance of the **idea** of an influenza virus, and has published these. However, **no virus was seen or isolated from a corpse nor was a piece of gene substance of a virus isolated.** By means of the PCR technique pieces of gene substance were **produced out of nothing whose earlier existence in the corpse could not be proven.** **If viruses** had been present, then these could have been **isolated**, and **out of them** their gene substance could have been **isolated** too; there would have been **no** necessity for anyone to produce laboriously, by means of PCR technique - with a clearly **fraudulent** intention - a patchwork quilt of a **model** of the genetic substance of the **idea** of a Flu virus.

Q…How can a layman check on this?

About these short pieces of gene substance, which in the sense of genetics are **not** complete and which **do not even** suffice for defining a gene, it is **alleged** that they together would make up the entire gene substance of a Flu virus. In order to see through this **Fraud** , one only has to be able to add up the published length of the pieces, in order to ascertain that the **sum** of the lengths of the individual pieces, which supposedly makes up the **entire** viral gene substance of the alleged flu virus, does **not** match the length of the idea of the genome of the influenza virus

model. It is even simpler to ask in **what** publication you can find the electron microscope photo of this **supposedly** reconstructed virus. There is **no** such publication.

Q…It is being **alleged** that these experiments have demonstrated that this reconstructed virus from 1918 would **kill** very effectively. What might be untrue in this?

If I inject into a hen embryo a mixture of artificially-produced pieces of gene substance and proteins and aim at the centre of its heart, then it **dies faster** than if I only inject the mixture into the embryo peripherally. If I expose cells in a **test**-tube to a quantity of **artificially**-produced gene substance and proteins, then they die **faster** , than under the standard conditions for cells dying in a test-tube, something which normally is being **presented as proof of** the existence, **as proof of the isolation** and **as proof of the multiplication of the alleged virus.** Based on this **artificially** produced genetic substance, which is **presented as viral, models of Proteins are being made with computers.** Proceeding from these protein models, the **image** of the **entire** virus is **reconstructed with computers.** That is all, **but the whole world believes that you can reconstruct viruses in laboratories.** Thus it's no wonder either that, referring to statements by the CIA and by the British secret service MI6, it is alleged that in North Korea, the communist regime would now be producing flu viruses even more

deadly than H5N1.

Q…What conclusions do you draw from this?

Since the head of the supposed al-Qaida, Bin Laden, has **not** been found, al-Qaida in **Arabic** only means -The Road- and **nothing** had been heard of this organization before the sudden destruction of the collapse-endangered skyscrapers in New York. Since, **just as the alleged smallpox viruses have not been found,** no weapons of mass destruction of Saddams were found, which were the reason for the second Iraq war, and **now once more some deadly viruses are being alleged to exist, it should be obvious** who in reality are the terrorists and who in reality are the suicide bombers: All who are participating in the **virus panic** and are contributing to it! In the pandemic **plans**, a possible breakdown of the provisioning systems and **of public order,** in connection with the **declaration** of a bird flu **pandemic**, has been envisaged by the WHO. **The estimates of up to 100 million deaths should be taken seriously. I see all inhabitants of homes for the aged as being the first under threat, who in an** outbreak of chaos and a breakdown of the Health care systems, and with that public order and besides them **small** children as the most protectionless **and** defenceless victims. It would be hardly possible to imagine, if these **fabricators** of contagious-disease would declare the **emergency** during the **winter**.

Q…Does then the substance Tamiflu, which is now being purchased with tax money and stock-piled, protect people from the bird flu?

Nobody is alleging that this substance protects against a Flu . Tamiflu is supposed to function as a neuraminidase-**restraining** agent. It **restrains** in an organism the function of the **sugar** neuraminidase acid, which is co-responsible for the surface tension in the cells. Those **side effects** which are noted on the instruction leaflets accompanying packages of Tamiflu are **almost identical to the symptoms of a serious Flu.** In large quantities thus, medicines are now being stored , **cause precisely the same symptoms as those which appear in an actual so-called Flu -** and which will abate with a doctor after seven days, and without a doctor after a week. If Tamiflu is administered to **sick** persons, then this is **likely to cause far more serious symptoms than those of a serious Flu. If a pandemic is stated to exist, then many people will take this medicine at the same time. In that case we will actually have unequivocal symptoms of a Tamiflu epidemic. Then deaths caused by Tamiflu are to be expected as well, and this will then be presented as evidence of the dangerous nature of the Bird Flu and evidence of how anxious the State is** that people should be in good health. In this, the **well-tested** AIDS **pattern** is being **repeated**.

In Spain it is noted on the instruction leaflets accompanying packages of AIDS medicine that **it is not known whether the symptoms are caused by the medicine or by the virus.**

Q…Then you will also not recommend any general vaccination or the specially developed vaccination against the bird flu?

I am not recommending any madness. Every Vaccine contains poisonous substances which have effects lasting a long time resulting in some or more serious injury. The infection protection law (in Germany) requires, as a necessary precondition , for a vaccination to be justified, the fact that a pathogenic **agent exists** , for instance a **virus**. Since **none** of the so-called disease inducing **viruses** can be, **nor** should be, alleged to exist, there can also be **no lawful** vaccinations against influenza, and none against bird flu either. Every vaccination which has occurred in Germany **after** the **infection protection law became operative** on 01.01.2000 is a **crime** of serious bodily assault. Of course I am not recommending people to allow themselves to become victims of law-breaking and crimes.

Q…What in your opinion lies behind all this which we are now experiencing here?

About this one can only speculate. Of course the pharmaceutical industry is happy about the big business with the bird flu panic. But in fact every individual is behind this madness. The situation is what it is. The situation could **only come to this because we as citizens have allowed our State to act in this way against people,** although our State is formally a democratic constitutional State. Anyone who waits for the pharmaceutical industry to do something for the good of people concerning this , will wait in vain. Anyone who does not defend himself, makes a huge error. Anyone can ask the Ministry for Consumer Protection, the Ministry for Public Health etc for **that scientific proof which would justify the bird flu panic.** Anyone who waits for the others to do something should not wonder if those others do nothing and the situation does not remain what it is but even gets much worse. **In the final instance we, the citizens, stand behind this, in that we for years without doing anything about it, have seen the whole madness around us and have tolerated it.** Here we must begin to take **social responsibility**, if we do not want to surrender and sacrifice ourselves to the total domination and chaos of an uncontrolled **Pseudo**-Science.

Q…Must then in your opinion science be combated?

The domination of the Pseudo-Science must be overcome

by means of a Social science which is characterized by its obligation to truthfulness and by the possibility to verify it and to control its actions. The language of the present university medical science , reveals that in it, an uncontrolled democratically-lawfully prevailing orthodoxy stands in the foreground, when the Orthodox Medicine Practitioners and the State are referring, as justification for their actions, **to the dominant opinion of medical science to which we supposedly have to submit even if** this prevailing orthodoxy maintains that the babies are brought by the stork. We have however no reason to complain. **It is us who tolerate this Governmental behavior.** Anyway, nobody should wonder, if he continues to accept that we must surrender ourselves to this prevailing orthodoxy. as we are doing now faced with the absurdity of the Bird Flu allegations, if he then wakes up one morning and with horror realizes that he is dead. Killed by that prevailing orthodoxy which he as a citizen of a democratic state built on law has tolerated. In a democratic state built on law, **the bird flu panic would be just as impossible as would** AIDS and vaccinations. We the citizens must create the constitutional state. Then not only AIDS but also the pseudoscience and the bird flu will have no chance. I can only say: Check things out! Use your reason!

Translated by: James McCumiskey, Rolf Martens and Susanne Brix

Dr. Stefan Lanka Exposes The "Viral Fraud" and: **HIV PICTURES; WHAT THEY REALLY SHOW** By Stefan Lanka and: No Panic. The Truth about Bird Flu, H5N1, Vaccines and **AIDS**

All these photos have in common that they, resp. the authors, **can´t claim that they present a virus,** as long as they do **not** also provide the original publications which describe **how and what from** the virus has been isolated. Such original publications are cited **nowhere**.

Indeed, in the entire scientific literature there´s **not** even one publication, where for "viruses in the medicine" the fulfillment of Koch´s first postulate is **even claimed**.

That means, that there is **no** proof that from humans with certain diseases , the viruses - which are held responsible for these diseases - have been isolated. **Nevertheless**, this is precisely what they publicly claim. Now, regarding the photos submitted: 1.**Fraud** => The **particles** are claimed to be HIV, but are cellular and **not** viral particles. The debris on the lower part of the photo indicates that the particles are **not** purified or isolated. **These photos are always published without any evidence that the particles are of viral origin.** 2.**Fraud** => HIV researchers **believe** the AIDS virus looks like this; like a bomb or water (blood) mine. This model is **based on** the detection of cellular particles in cell lines **under very**

special conditions. Such particles **never** have been isolated or somehow else demonstrated to exist **as a virus or be of viral origin.** 3.**Fraud** => Computer enhanched EM photo of a cell surrounded by small particles. The blue/grey stained particles, which are **claimed** to be HIV particles attacking or (**depending** on the publication) leaving a white blood cell, are **artefacts** of the staining and/or fixation process and **at best may be** cellular particles entering or leaving the cell. These kind of nice photographs, made by **Lennart Nilsson**, have been published **without** any evidence that the particles have a **viral origin**. 4.**Fraud** => The images of the so called **HIV**-, **measles** (Masern)- and **smallpox** (Pocken) viruses clearly show, that these are cells wherein the viruses can **allegedly** be found. **Thus, nothing has been isolated.** The photos actually show cells and typical endogenous particles in them. These structures are well known and serve the intra- and intercellular transport. **Unlike** viruses of the same kind - that are **always** the same size and same shape (consistency) -, they **differ** in size and shape (consistency) and therefore **can´**t be isolated. 5.**Fraud** => In the case of the **influenza- herpes-, vaccinia-, polio-, adeno- and ebola-viruses** each photo shows **only** a single particle; **nobody claims that they´re isolated particles,** let alone particles that have been **isolated** from humans. 6.**Fraud** => Fraud => The **"isolated" polio viruses are artificial particles, generated by** suction of an indifferent mass through a very fine filter into a

vacuum. Its structure (no characteristic structures) **differ** clearly from the ones of the "viruses" in the cells. Here the information is **essential** that a biochemical characterization of those "isolated" viruses, although "isolation" is **claimed**, has **never been published anywhere nor has anybody even claimed such a characterization.** 7.**Fraud** => Fraud => Fraud => Fraud => The photo of the hepatitis B "viruses" does **not** show isolated structures, but an agglutinate. This is the scientific/medical term for proteins from the blood that are clumped together, as is typical for coagulations. Typically, thereby round and also crystal structures accrue - depending on the condition of the blood sample. 8.**Fraud** => H5N1 (= Hoax 5 Nonsense 1) If one hen lays fewer eggs **or** gets a blue crest and that hen is tested H5N1-positive too, **then all the other hens are gassed.** That is **how** the apparently 100 million killed hens from H5N1 was **achieved**. 9.**Fraud** => H1N1 (= Hoax 1 Nonsense 1) People should be smarter than hens. **Why** do they say H1N1 is a mixture of **different** viruses and make us suspect, it must have been made artificially in some military-lab? I guess they only try to make people **believe** in the thread. Don't trust World Hoax Organization -**WHO**.

Some Statements from: Dr. Stefan Lanka Exposes The "Viral Fraud".

For almost one year we have been asking **authorities**, **politicians** and **medical institutes** , **for the scientific evidence for the existence of such viruses that are said to cause disease and therefore** require "immunization". After almost one year we have **not** received **even one** concrete answer which provides evidence for the existence of those "vaccination viruses".

The conclusion is inevitable that our children are still vaccinated on the basis of scientific standards of the 18th and 19th century. In the 19th century **Robert Koch** demanded in his generally accepted postulates evidence of the virus in order to **prove** infection; at Koch´s time this evidence **couldn´t** be achieved directly by visualization and characterization of the viruses, because adequate technology **wasn´t** available at that time. Methods of modern medicine have profoundly changed over the past 60 years, in particular by the invention of the **electron** microscope. **And still all these viruses we get immunized against have never been re-examined using this technology?** Dr. Stefan Lanka, virologist and molecular biologist, is internationally mostly known as an "**AIDS** dissident" (and maybe "gentechnology dissident") who has been **questioning the very existence of** "HIV" **since 1994.** In the past years, however, he stumbled over a breathtaking fact: **Not even ONE of the (medically relevant) viruses has ever been isolated; there is no proof of their existence.** Actually, Dr. Lanka has already

stated three years ago, in the almost "legendary" Zenger´s interview: "So for a long time I studied virology, from the end to the beginning, from the beginning to the end, to be absolutely sure that **there was no such thing as HIV.** And it was easy for me to be sure about this because I realized that the **whole group** of viruses to which HIV is said to belong, the **retroviruses -- as well as other viruses which are claimed to be very dangerous** -- in fact **do not exist at all.**" So he was thoroughly reading the literature on those "**other** viruses" again, and after he could still **not** find any paper which would provide the evidence, he encouraged people **not** to believe him but to ask the institutes and authorities themselves. This has actually taken place, mostly initiated by mothers. The responses were revealing. In September 2001 the German book "Impfen - Völkermord im dritten Jahrtausend?" (**Vaccination - Genocide in the third millennium?**) by Stefan Lanka **and** Karl Krafeld was published in which they state that **there is still no proof of any (medically relevant) virus.**
https://www.psitalent.de/Englisch/Virus2.htm

The Truth about Bird Flu, H5N1, Vaccines and AIDS Dr. Stefan Lanka Exposes The "Viral Fraud"
http://educate-yourself.org/cn/stefanlankaviralfraudexposed04may09.shtml

Pictures of "Isolated Viruses" Debunked.

What all of these photos have in common is that they, according to authors Stefan Lanka and Karl Krafeld, **can´t claim that** they present a virus, as long as they do **not** also provide the **original** publications which describe how and from what the virus has been isolated. Such **original** publications have been cited **nowhere**. Indeed, in the entire scientific literature, there´s **not** even one publication where Koch´s First Postulate is even **claimed** for "viruses in medicine" .

That means, that there is **no** proof that from humans with certain diseases that the viruses - which are held responsible for these diseases - have been **isolated**.

Nevertheless, this is precisely what is publicly claimed.

Interview with Dr. Stefan Lanka, Ph. D Challenging BOTH Mainstream and Alternative AIDS Views.
.. By Mark Gabrish Conlan for Zenger's Dec. 1998
http://www.virusmyth.com/aids/hiv/mcinterviewsl.htm

While most people in the U.S. and Western Europe go right on **believing** that the so-called Human Immunodeficiency Virus [HIV] is the **sole cause** of AIDS, debate rages even within the alternative AIDS community over whether HIV **exists at all.** Though **Peter Duesberg,** Ph.D. -- virtually the only alternative AIDS

theorist with any significant public reputation -- **continues to insist that HIV exists but is harmless, other** alternative AIDS researchers and activists are coming to the conclusion that **the virus doesn't exist.** The main proponents of this view are **Australian** researcher Eleni Papadopulos-Eleopulos and her colleagues, who argue that HIV has **never been isolated according to the Pasteur Institute criteria of 1973,** and therefore it's probably what's called an "endogenous **retrovirus**" -- a **creation of the body's own** genetic material that looks and functions partly like a **virus ?**, but is **not** an infection because it comes from the body's own cells. **Stefan Lanka, Ph.D. takes the challenge to HIV's existence even further.** A German researcher, Dr. Lanka is usually referred to as a virologist. But that hardly begins to describe his wide-ranging fields of study. Based on experiences in marine biology, biochemistry, evolutionary biology and virology, he's worked out a whole new view of HIV and AIDS. He says that **all so-called retroviruses are actually the body's own creations;** that hepatitis is an autoimmune disorder (a disease in which the body is attacked by components of its own immune system) rather than a viral disease; that AIDS has **nothing** to do with immune suppression; and that it should really be called Acquired Energy Deficiency Syndrome -- AEDS -- because its **true cause** is a breakdown in the delivery of oxygen to the blood and/or body tissues. Dr. Lanka did a West Coast tour in October and spoke to H.E.A.L.- San

Diego on October 20. Zenger's interviewed him hours before that event.

Zenger's: I'd like a little about your background, what your training is, when you studied, what you specialized in, and essentially how you came to these ideas about AIDS.

Dr. Lanka.: I started studying molecular biology in 1984, and I soon got bored because I learned that all that you have to learn in order to pass the exams is already old, out-of-date dogmatic thinking. So I went into ecology because I realized, while being abroad in different countries, that you can carry out very important research without big machines or big money. I was looking for an opportunity to do molecular genetics in the field of biology, so I chose to move into marine biology and did a lot of **electron** microscopic studies. A marine biology professor was willing to let me work for him, and while doing this I found a stable **virus**-host relationship by accident. In that very moment, I knew that was it. The best way to do meaningful genetic research is to have a stable **virus**-host relationship, in which a **virus** is produced in the host but does not kill the host. So you can really study how they interact, how the genetic material of the **virus** is produced and how it interacts with the host, without manipulating it. That's **still the only stable virus-host** relationship in virology, other than in bacteria. I was

glad to be able to carry out this study, but first I had to convince my professor so he would agree to finance my new studies. He said he was a classical biologist and he could not sponsor me as a researcher in virology. I needed to find another professor who was willing to guide me, and the very day I found one , I got a lab of my own. I could buy all the tools and big machines on my own overtime, so I had the best conditions to start my studies. After one year, I had **isolated** a virus and characterized it. When I started doing **viral** research, it was already 1986, 1987, just when the public in Germany and Europe was starting to become **aware** of AIDS. Because AIDS was supposedly caused by a **virus**, I was automatically considered a specialist in the AIDS field. In the beginning, this was a nice feeling. I was telling people what I heard **from** the **mass** media and the TV, and I was **not** checking the **evidence because everybody was convinced** AIDS was a viral disease. Then I heard about the things that **Robert Gallo** [American cancer researcher who first identified HIV as the cause of AIDS] was doing **wrong**, and that he was **misleading** the public about his **first retrovirus** [HTLV-I, which Gallo **claimed** to be the cause of AIDS in 1982, before his **alleged** discovery of HIV] and he had **stolen ???** the virus from **Montagnier**, and all this kind of gossip. I already had a somewhat critical attitude when I started studying molecular genetics, so I went to the library to look up the **literature** on HIV. **To my big surprise, I found that when they**

are speaking about HIV they are not speaking about a virus. They are speaking about cellular characteristics and activities of cells under very special conditions. I was so deeply shocked. I was thinking, "Well, I'm not experienced enough. I have overlooked something. On the other side, those people are absolutely sure." Then I was afraid that speaking about this with my friends, or even my family, they would think is absolutely mad and **crazy**. So for a long time I studied virology, from the end to the beginning, from the beginning to the end, to be absolutely sure that there was **no such thing as HIV.** And it was **easy** for me to be **sure** about this because I realized that the **whole group of viruses to which HIV is said to belong, the retroviruses** -- as well as **other** viruses which are claimed to be very dangerous -- in fact **do not exist at all.**

Zenger's: So it was just on the basis of this reading that you concluded that what is called HIV, what is considered to be the "HIV virus" and is supposed to be infectious like other viruses that are acknowledged pathogens, really represented a phenomenon within the body. How did you figure that out, and why are you so sure about it?

Dr. Lanka: I was wondering what viruses are for in evolution, because they **didn't** seem to have any function other than to be very dangerous and killing other cells. So

I went into evolutionary biology and found that **the first ge**netic molecule of life was RNA, and **only later in ev**olution did DNA come into existence. Every one of our genomes, and that of higher plants and animals, is the product of so-called **reverse transcription:** RNA transcribed into DNA. But I had already realized by then that the thinking about molecular genetics was very **dogmatic**. In the early 1960's they came up with the **central** dogma of **molecular genetics,** which try to uphold even today, and which is **ridiculous**. The dogma says that DNA behaves in a **static** way; DNA makes RNA; RNA **cannot** be transcribed back into DNA; RNA comes into existence **only** on the basis of DNA. That was and is the basis, of the central dogma of molecular genetics. **I found that this kind of thought came from research funded by** the seed-producing industry of the United States, and that a whole body of existing knowledge -- namely, that of cytogenetics, before World War II -- was just **suppressed** or **even** slandered as "lazy science" **because it had been carried out mostly in Europe.** This kind of science well established that the genetic material is **not stable**. It is subject to change, and this means the genetic material is **reverse-transcribed.** It goes in **both** directions. This **earlier** research also established that inside the cell we have a huge amount of genetic material **other than** that of the nucleus. But because **molecular genetics and molecular biology were actually founded by physicists,** who thought they could

explain the whole structure of the atom just by focusing on the nucleus, when they went into biology they carried over that **same** mistake. They focused **only** on the nucleus of the cell and **claimed** it was responsible for all of how life comes into existence, how it's controlled, etc. This is **ridiculous**, because they have **overlooked** the **essential** of life: the production of energy. While studying the evolutionary aspects of biology, I quickly realized that **reverse** transcription is common to all forms of life, and in fact is the **basis** of all higher living. **Later** I learned that **reverse** transcription is a **repair** mechanism for chromosomal DNA. But the mainstream of molecular genetics is still committed to the central dogma: **"There is no such thing as reverse** transcription from RNA to DNA." In 1970, when they detected biochemically that **there is a reverse** flow of genetic material, they **didn't** give up the dogma or even try to change it. Instead, they called it an **exception** to the central dogma of molecular genetics, and **explained it by postulating the existence of retroviruses.**

Zenger's: Excuse me, but I thought that the field of **retrovirology** had started as far back as 1911, with Peyton Rous and his experiments with chickens. [Rous surgically removed cancerous tumors from chickens in his lab, ground up the tumors, fed them to healthy chickens and observed that the healthy chickens who ate **ground-up tumors** grew tumors themselves. He **concluded** that

the tumors **may have been caused by** an infectious agent being transmitted from the sick chickens to the healthy ones.]

Dr. Lanka: No, it was only in retrospect that he was cited as the one who was dealing with **retroviruses**. What Peyton Rous actually did was he **inbred** his animals so heavily that the genetic material from the different strains he used to breed became more and more **similar**. When the animals' genetic materials become **too similar** to each other, then **even more** genetic material is **interchanged** between the chromosomes than happens normally. Often, in inbred animals or plants, on two places of the chromosomes genetic material in between **got** lost. Then you will see the characteristic chromosomal damages in inbred animals, plants or human beings, resulting in disabilities which are well studied. So, because Rous's chickens were so **heavily** inbred, they had a high rate of spontaneous cancer induction. The **results from this research were not cited for more than 20 years.** Later, some people tried to **speculate** about them. In the late 1960's and early 1970's they started to think about this because molecular biology **took over** modern medicine, and argued -- **against** the existing body of knowledge, of facts -- that cancer is **caused** by infectious entities: by **viruses**, or mutations, or **viruses** causing mutations. They ignored the fact that cancer has something to do with oxygen deficiencies, which had already been established

by Otto **Warburg's** research. Warburg had received his first Nobel Prize demonstrating how a cell is able to produce much more energy than in the process of fermentation, using oxidative respiration. And he had received his second Nobel for proving that cancer is characterized by the process of fermentation; that oxidative respiration is not taking place in cancer. And this has been just ignored. So in 1970, when they proved that **reverse transcription does happen** and they discovered the enzyme, **reverse transcriptase,** which does it, they **wouldn't** give up the dogma. They changed it slightly and said there is an **exception**; and that it was associated with the existence of a **new** class of **viruses** called **retroviruses**, **which they cannot prove exist in other ways.** When I was absolutely sure about everything I've told you so far, I went public. I was invited to a **lot** of conferences on marine biology and biology, and at every conference I presented my own data. I used every opportunity to speak out **against** HIV, and I quickly learned that because I was taking away HIV as an explanation for AIDS and was **not** able to replace it with something else, and **not** being able to explain what's going on under the label "HIV," it forced me to watch out and find those people who were able to explain what's going on. In the beginning, of course, some of the publications of Peter **Duesberg** helped me a lot, because he was an authority who questioned a **lot** of things, and that helped me. I translated some of this articles into

German and published them in a small publishing house. But then, with time, I learned about **other specialists,** among them **Heinrich Kremer,** the well-known German medical doctor, former medical director of the Federal German Drug Abuser Clinics, who helped me to understand what was really going on. Because he was in charge of the introduction of **hepatitis B vaccine** into Germany, and used it in his patients, Dr. Kremer checked out the hepatitis B vaccines on the market. He found that the American vaccine, hepatitis B vaccine, was produced with the sera donated by men in the **Gay** scene in New York City between 1978 and 1980. So, as he knew, there was a lot of sex going on in a minority of these men, and therefore they had had a **lot** of sexually transmitted diseases. So he was afraid of using this vaccine, and instead he used the **French** vaccine, which was produced from blood donations by the **general** population in France. But in 1983 the **German** government **forced** him **not** to use this vaccine anymore. They said the French vaccine is **poisoned** by the "AIDS virus" -- at the time when **nobody** was positively speaking about an "AIDS virus" -- **but** the American vaccine was O.K. He knew, or he was warned, that this had **nothing** to do with the science, but it had to do with the **fact** that **the German medical system, in parts of Germany, is virtually a colony of the American system.** Soon after, in 1984, he was told to deliver frozen blood samples of his patients to Berlin, to the newly founded AIDS Center, to be tested

for the "AIDS **virus**." Before he let his blood out, he checked what's the **evidence** for the accuracy and reliability of the HIV **antibody** test, and he realized that this test is **not** able to detect the virus. It is **not** able to say yes or no, you **are or are not** infected. It is **only** able to say that you have a higher or lower amount of antibodies. That's **how** the HIV antibody test **was and is** designed.

Zenger's: It's my understanding that when you have an antibody test that is actually useful, like the antibody test for **syphilis**, you get a high or a low antibody reaction, and it's a certain multiple of how many times you **dilute** the original sample and still have the reaction. Therefore you know **not** only that the **infection** is present, **but also how well** the immune system is responding to it.

Dr. Lanka: I'm absolutely sure that **no antibody test in medicine has any absolute meaning. Especially** in HIV antibody testing, it is clear that the antibodies that are detected in the test are present in **everybody**. Some people have them in **higher** concentrations, and some in lower concentrations, but **only** when you reach a **very high level** of antibodies -- much higher than in any other antibody testing -- are you considered to be "positive." This is a **contradiction** in terms because in **other** antibody tests, the **lower** your level of antibodies, the higher your risk for a symptomatic infection. But with HIV they say you are "positive" **only** when you have

reached a very high level of antibodies. Below this level, you are said to be negative.

Zenger's: So this is what Dr. **Roberto Giraldo** was talking about when he spoke to H.E.A.L. in San Diego. He said that when they do the HIV **antibody** test they **dilute** the sample to 1/400 of its original strength, and if they **didn't** do that **all the samples would test positive.**

Dr. Lanka: That's it. How **ridiculous**. Dr. Kremer knew this already by 1984. He was very worried about the fate of his patients, because in 1984 the **politicians** asked him to put these already **stigmatized** "HIV-positive" patients into **quarantine**, which means to separate them from the other ones. He said **no**, because there's no infectious entity out there. He knew everybody **who went through chronic active hepatitis or had the hepatitis B vaccine would test** "HIV-positive." So he knew that there is **no** infection in his hospital. He informed the mass media, who went to his hospital to inform themselves, in great detail. He told them all the evidence. And the very same journalists, in talk shows, in Der Spiegel [one of Germany's **largest and most popular** magazines] for example, published just the **contrary**. So he knew that it was **intentional** from the very beginning. They played **war**. They **all wanted to have a blood and sex plague, contrary to the evidence** which he presented to them. So he knew that AIDS was **built up on** misconceptions. He

was dealing at the **top** political level. They told him, **off the record, that they knew, they didn't care**. They even tried to **kill** him, and this didn't succeed. He had a good intuition and got out of his car before the tire blew out. Then he learned from a minister who had a deep respect for him, because of his work with prisoners and drug abusers, that the **German** government was carrying out a secret psychological investigation, trying to prove that he was **mentally** ill and being kept in his job only because they considered him in danger of committing **suicide**. So when he learned this, he left his very highly-ranked position because he was not able to be silent on this. That would not fit his ethics. I also met Professor **Alfred Hässig of Switzerland.** He founded Swiss blood-donation system and was **one of the first** to take out products from the blood in order to make plasma to treat chronic disease. By becoming a colleague and a very close friend of his by now, I learned a great deal about the whole **blood**-producing **industry** and the criminal energy behind it. In March of 1996 in Berne [capital of Switzerland], Hässig, Kremer and I met for the first time. It became clear, also, what's happening in the field of **hepatitis**. They are **not** dealing with a virus. Of course, there's a possibility to enrich certain kinds of proteins in blood products, which then **cause** severe autoimmune reactions, **but only in very stressed-out people, never in non-stressed people.** When they learned to take out these proteins from the blood products, or **dilute** them,

there are **no** hepatic problems anymore. I learned this through him.

Zenger's: Are you saying that all forms of hepatitis are non- infectious, or just some of them?

Dr. Lanka: No, there's **no such thing as infectious hepatitis.**

Zenger's: So there are **no hepatitis viruses,** either?

Dr. Lanka: Yes. Hässig was always fighting to make sure that blood products were produced **only** on the basis of a small pool of donors who were young and healthy. The industry started to produce blood products on the basis of commercial blood donations, using a huge amount of blood samples, pooling them all together in a large pool, because then it was much cheaper to get out all the various kinds of products.

Zenger's: In this country, it gets even worse because blood donations are one of the principal ways homeless people have of staying alive. As a result, we're taking a lot of our blood supply from people in society who have the least healthy lifestyles.

Dr. Lanka: I know all the details. This what I'm going to tell you. Professor Hässig once met the person responsible

for the industry to produce **industrial** blood products, and once, when this person was **drunk** while visiting the Fiji Islands after a conference in Australia, this person told Professor Hässig that soon they are going to smash the state-owned blood producing units, based on voluntary blood donations, because they're much cheaper producing their blood products because they go into the **Third World** countries, and they are already there in all the **prisons** of the dictators in South America and elsewhere. When Hässig heard about this, he rang some of his friends -- and, of course, **Hässig** was the **leading** person in the **blood business** -- and at this time there were some non-corrupted people in the WHO (World Health Organization). So, in an emergency meeting, on short notice so the industry had not time to corrupt the members who decided on these issues, they decided that the position of the WHO would be that it isn't allowed to produce plasma in the Third World, **because** they would bleed them out. Now they are **bleeding out the poorest of the poor,** and they are going to Mexico, near where we are sitting right now. In order to help the commercial blood products **industry**, the FDA [U.S. Food and Drug Administration] has **approved that a single person may give up to 50 units of plasma a year.** That **means he may drop in two times a week** to give blood and **liver** plasma. And an elephant wouldn't possibly survive that, right? So that's the background, and what they did when all that was in place was they changed the way they were

treating **hemophiliacs**. It started in California. Up to the year 1969 it was **forbidden** to give the clotting factors to hemophiliacs **unless** they had internal bleeding. If they would give them prophylactically, **antibodies** would be produced because these blood products are **highly contaminated.** In 1969 the industry started to convince some medical doctors -- and the **first** one was a **woman** doctor in California -- to treat hemophiliac patients prophylactically with those clotting factors, and this is how the industry made a lot of money. And, of course, the bodies of these hemophiliacs made a lot of **antibodies** against those products, which had been foreseen. They've had to use **higher** doses of clotting factors ever since, in order to **compete** with those antibodies, **so that those clotting factors actually work.** They gradually have to **increase** the amount they are injecting. This has been the **biggest** business in the blood industry ever since. Nobody's speaking about this, **but that's why almost all hemophiliacs have come down with hepatitis.** If you inject such a high amount of **foreign** proteins, and **all** the contaminants, then of course the liver, as the central metabolic organ, is **stressed** out, resulting in hepatic inflammations. A lot of hemophiliacs **died** from hepatitis, and it was **blamed on nonexistent viruses.**

Zenger's: One of the issues that's raised in groups whenever we're talking about the theories that HIV doesn't exist, or that **retroviruses** don't exist, or that this

or that disease isn't infectious at all, is we often get people saying they're having a hard enough time just trying to get people to think that HIV might be harmless. It would be way too much to try to convince them that it doesn't exist at all, and even more difficult to try to convince them that -- if I understand what you're saying correctly -- ever since the end of World War II virtually **every** scientist working in this field has been **absolutely committed to a totally wrong theory and** that all of that research is **nonsense** and has to be thrown out.

Dr. Lanka: That's not true. Before AIDS, there were a lot of discussions and papers about the role of **viruses** in evolution. Evolutionary biologists were already arguing about the central dogma of molecular genetics. But this was all **silenced**, because they all experienced **how rapidly that idea came into existence,** and how powerful it was. Even when some of my colleagues at the university and everybody I reached was absolutely sure and clear and convinced about what I was saying, they were **silent**. I never got support from a lot of professors at my university. Some of them, of course, liked me a lot and they tried to warn me when it was too much, when I was in danger of being expelled from the university, etc. But none of them went public on their own.

Zenger's: When would you date the beginnings of this mistake, what you call the dogma? How long has it been the dominant paradigm?

Dr. Lanka: I think it really started in the 1960's, when the **retrovirologists** were being supported by President Nixon in the "War on Cancer." This was the first time **incredible** amounts of money were poured into this kind of research. These **elite schools of thought** came into existence, dominating everything, and of course they had success with the mass media because they were dealing with cancer. When they **claimed** that retroviruses were the cause of cancer, of course they developed **chemotherapy** against it. But soon they had to **give up** the idea of cancer being caused by **viruses** because they saw that **reverse transcriptase and reverse transcription occur everywhere they look for it.** They found it's a **common** characteristic of all forms of life, **especially** for cancer cells, and in fact it's a **repair** mechanism. So silently, slowly but surely, they **stopped** speaking about those cancer-causing **viruses** anymore, but came up with a completely **new** idea of what is causing cancer, **saying it's a weak** immune system. When **immunology**, as its own biological discipline with is own faculty came into existence, people **claimed** that they were able to measure the **strength** of the immune system by measuring **lymphocytes** in the bloodstream. Of course, **thousands** of studies had been carried out in the '70's saying that the

white blood cell count never correlated with any disease or with any age. But, even so, they **claimed** that cancers come to existence by **accidental** mutations everywhere in the body, **and** the immune system is suppressing cancer. And when the **T4** cells are out of order with something else in the immune system, the immune system cannot suppress cancer anymore. And this was the **immune surveillance theory** of cancer, which was **wrong** already at the moment they announced it; because they **knew** already by then that cancer cells **have no specific markers on their surface.** They have the **same** protein markers on their surface as embryonic cells.

In order to explain failure to find a retrovirus that directly caused cancer, they claimed to be able to measure the immune system. But this is ridiculous.

In the Journal of the American Medical Association, August 28, 1981, it was published that it makes no sense to measure lymphocytes in the blood because only a few of them are in the blood.
The immune system is carried out, **not** in the blood, but in the tissues. **Only rarely and accidentally do we see some of them in the blood.** We've already carried out **thousands** of studies which have **proven no correlation between disease or health, in old or young, in** T-cells; and even less, of course, in T-cell subsets. But, even

though they **knew** that these T-cell tests had **not** meaningful , they were selling them to the market.

Beginning in 1977, starting in the United States, it was **possible to patent biological entities or biological techniques,** so people started to make money out of biological ideas. This is the definite turning point when modern medicine and modern biology lost their 'Unschuld', their innocence. That's it. The immune surveillance theory of cancer -- the belief that if you measure the **strength** of the immune system, then you could see when you are going to develop cancer -- was the **basis** of AIDS, the thinking about AIDS. They said if your immune functions are weak, you are going to develop **all viral** forms of opportunistic infections and all forms of cancer. And this **never** happened, as a matter of fact. In AIDS we **never** have seen opportunistic infections. We have **never** seen all viral forms of cancer; **only one form of cancer,** KS [Kaposi's sarcoma].

Zenger's: When you say, "In AIDS we have **never** seen any opportunistic infections," what do you mean by that? Because virtually **everything** associated with AIDS is considered an "opportunistic infection."

Dr. Lanka: That's **not** true. An opportunistic infection is a **bacterial** infection which takes over when the immune functions are down, when you have an immune defect or

an immune deficiency. This **was and is** the definition of an immune defect, and an immune deficiency: when bacterial infections are taking **over** in your body, **generalized** bacterial infections. This is the case in those children born with an immune defect, who have to live under a plastic tent; or those people in the intensive- care units, patients dying now like **flies** because they are having immune deficiency after an operation, accident, **transfusion** or transplantation, when immune functions are **artificially** suppressed. Bacterial infections go everywhere in the body, and due to the resistance catastrophe, which is the medical background of why "AIDS" has been **invented**, definitely, they are dying like flies. But all these **internalized** bacterial infections **never** have been part of the **definition** of AIDS.

Zenger's: I remember that was a question the AIDS experts got asked at some of the very early meetings, in the early 1980's: "Well, **if it's a breakdown of the immune system, why don't you get** colds all the time? Why don't you get flus all the time? Why don't you get these common infectious diseases all the time? Why is it just these really **esoteric** things like PCP and KS and CMV and MAI and whatever?

Dr. Lanka: That's it. The **only** diseases seen in people with AIDS are the **ones** tropical disease specialists have specialized in. …..

Zenger's: Which is why, if you're growing **mushrooms**, you put them in a warm, **dark** place and fill them full of pieces of wood and bits of plants.

Dr. Lanka: That's it. It was already known by 1965, definitely, that PCP is a fungus. And this **was and is** the most important AIDS-defining disease. If you look at who comes down with this disease, you see people who are using poppers. What are poppers? **Nitrites**. And check every dictionary in the bookstore, or the People's Medical Dictionary: what do nitrites do in the body? They oxidize the blood. So, of course, the first cells to suffer are cells in the **lung**. Nitrites are transformed immediately into nitric oxide in the smallest capillars [capillaries?, F.C.] of the body. Nitric oxide is produced **by the body in very low** concentrations in order to control blood pressure, in order **to control** development. It has to be detoxified by the body immediately, because in higher concentrations it acts very **aggressively**, destroying everything. This is why the "eating cells" of the immune system, the macrophages, are releasing nitric oxide in high quantities in **inflammation** reactions: to destroy and digest the bacterial cells. So if you take up nitrites regularly, or from time to time -- which means huge, excessive amounts of nitric oxide are produced -- it means you start the **self-destroying** process in your own body, especially in the lungs. You are destroying your lung tissue, and fungal

infections are growing on this dead organic matter. Even so, immune functions are **perfect**, because these patients do suppress bacterial infections. **All** those 60 different kinds of lung disease we know by now, **all** caused by bacterial infections, do **not** appear because the immune functions are still well. So we have a direct toxic effect, which may happen **even when** your detoxification system is **not** working on a cellular level, because you will suffer malnutrition. PCP can **also** happen in people who suffer **extreme malnutrition,** like we've had in Africa. This is the reason why PCP is **not** part of the AIDS definition in Africa, because we have it in the children who suffer **starvation** because the detoxification system of the cells is **very weak** in children. This is **why**, in the Middle Ages, when the **wells** had been poisoned by feces or meat from the civil wars or wars, it was **the children who suffered,** turning blue -- this was called "the disease of the blues" -- when they drank water, because there were a lot of nitrites **and** nitrates inside, produced by nitrifying bacteria when the wells had been poisoned, because the detoxification systems of children are very low. This is why the children **starving** heavily in Africa come down with PCP ever since. I can foresee, here and now, that people regularly using **Viagra** will be coming down with KS in two to **three** years because Viagra **acts by blocking** the neutralization of nitric oxide. When you take Viagra, nitric oxide accumulates, relaxing the smooth muscles, that blood is flowing in, the penis is being

erected, **and** our muscles are relaxed. Poppers act by the **same** mode, because the nitrites are transformed into nitric oxide in the smallest vessels, and so the smallest vessels become relaxed. But whereas poppers **directly** produce nitric oxide, Viagra works by **preventing** the neutralization of nitric oxide which comes into existence normally in the process of blood pressure regulation. It constantly persists at a very low level, **but if it accumulates, you are in a very big danger.** So, **if** the blood has oxidized itself and the **lining** of the smallest vessels, the capillars (i.e. capillaries, F.C.] , is destroyed by nitric oxide, what's going to happen? Those cells will **turn** into cancer cells. There's a lack of oxygen, and the first cells to suffer this oxygen deficiency are the lining of the epithelium, the smallest vessels, where the nitrites are transformed into nitric oxide. And this is, as a matter of fact, the **definition** of Kaposi' Sarcoma: when the lining -- the interior of the smallest vessels -- develops into cancerous form, growing bigger and multiplying. This is **hyperplasia**, **not** a form of sarcoma, but a real form of cancer, and this is **defined** as KS. It can also come into existence even if you are **not** swallowing poppers, but when your cellular **detoxification** system is **not** working anymore.

Zenger's: So that's your bottom-line answer to the question, "What is AIDS?"

Dr. Lanka: Yes. AIDS is an energy deficiency problem. The "AIDS" term is absolutely **misleading** because it has **nothing** to do with an immune defect or immune deficiency. It is clear that we are dealing with an energy deficiency. So the term "AIDS" has to be replaced by the term "AEDS," "Acquired Energy Deficiency Syndrome," and we would keep up the term "AIDS" **only** in the form of acquired **intelligence** deficiency syndrome. AEDS has a **rational** basis, and it is **treatable**. There are very potent treatment options available to **reverse** those damages caused by intoxification or lack of oxygen, on all various levels. Animal beings are not able to produce three major classes of substances. Among these substances animals cannot produce on their own are the **polyphenols**, which are vitamins. We are aware of 5,000 different kinds of polyphenols produced in **herbs -- in all plants, but especially in herbs.** The higher they grow, the higher they produce polyphenols. You can detect plants in front of radiation. These polyphenols are **nature's own protease inhibitors,** by the way. Animals are also not able to produce the **long-chain sugar** molecules which make up the basic tissues that form up to 80 percent of our body weight. These tissues produce the constant milieu for the cells in the body -- and if you don't have them you are going automatically into disease. Every cell is surrounded by these basic tissues, long-chain sugar molecules with **proteins** attached. All nerve cells end there, activating and deactivating. All immunological

reactions are carried out there. These basic tissues have a quasi-crystalline structure and they work by breaking, oscillating, very quickly, several thousand times a second, with the speed all biochemical reactions are triggered, etc. etc. If you don't know how life is working on the cellular level, you're not able to understand cancer. If you don't know how life is organized on the tissue level, you cannot understand life either, right? So if the cell **lacks** these substances, it cannot maintain its milieu. The surfaces of the cells especially need those long-chain sugar molecules in order to prevent **calcium** from flowing inside the cell. **If** those products are not there, calcium is formed inside the cells, **killing** the cells, resulting in **controlled cell death, apoptosis:** that means **inflammation?**. Normally you get these substances from **plants**. In emergency cases, if you are **depleted**, you get them from **bovine cartilage or agar agar,** two spoonfuls every morning, With this you can stop all forms of **arthritis**, by the way, And those molecules are **potent protease inhibitors** as well. In any case of **inflammation**, or **catabolic** situation -- when you lose more cells than the body's able to reproduce -- you go in with this and it's going to help you. The **artificial** protease inhibitors only help you for short periods. Then they **intoxify** the cells, because the **artificial** protease inhibitors **cannot** be digested. The body **cannot** get rid of them.
They form crystals, and eventually they intoxify the whole cell and the whole organism on all levels, because

they prevent the digestion of all the proteins. We have reached the end, with the treatments, because **not only are** we deconstructing AIDS and **offering** another term, which everybody's able to handle and be happy with, especially cancer specialists. We are also offering very potent treatment options to **replace** these very dangerous protease inhibitors. I think that completes the picture of what so-called "AIDS" really is and what you can do about it.
http://educate-yourself.org/cn/stefanlankaviralfraudexposed04may09.shtml

Pitfalls of Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction
Stephen A Bustina and Tania Nolanb 2004

The **transcriptome**, is context-dependent; i.e., the mRNA complement and level varies with physiology, pathology, or development. This makes the information contained within the transcriptome intrinsically **flexible** and **variable**.
If this variability is combined with the technical limitations inherent in any reverse-transcription (RT)-PCR assay, it can be difficult to achieve not just a technically accurate but a biologically relevant result. Template quality, operator variability, the RT step itself, and **subjectivity** in data analysis and reporting are just a

few technical aspects that make real-time RT-PCR appear to be a **fragile** assay that makes accurate data **interpretation difficult**.
It is important to recognize the considerable pitfalls associated with transcriptome analysis, with the successful application of RTPCR depending on careful experimental design, application, and validation.

Reverse-transcription polymerase chain reaction remains **the most sensitive** technique for the detection of often-rare mRNA targets, and its application in a real-time setting has become **the most popular** method of quantitating steady-state mRNA levels.

- **The most sensitive & popular.. doesn't mean perfectly accurate.**

However, it has also become clear that while the use of real-time assays has addressed some of the problems associated with conventional, gel-based RT-PCR assays, it has **also introduced new challenges** that must be appreciated and dealt with, **if data are to be reported in a biologically relevant way.**

Areas that require critical consideration are the **standardization** of quantitative RT-PCR (qRT-PCR) protocols3; attention to and **consistency** with regards to **reagents** used4,5; and the careful consideration of **assay**

design, template preparation, and analytical methods.6 This latter point, which includes the analysis, reporting, and **interpretation** of real-time data, is of particular importance when the aim is the quantification of **very low copy number targets -** for example, when extracting mRNA from **tiny** biopsies such as those derived from colonoscopies, single cells, or laser-capture microdissected samples. Unfortunately, in these circumstances, qRT-PCR data may be used in an **inappropriate** manner **to support conclusions that are not reliably related to the actual results obtained.**

Unlike DNA, which is as tough as old boots, RNA is **extremely delicate once removed from its cellular environment. Therefore, its purification is much trickier** than that of DNA and a template suitable for inclusion in an RT-PCR assay **must fulfill the following criteria:** It must be of the **highest** quality if quantitative results are to be relevant. It should be **free** of DNA, especially if the target is an intronless gene. There **must** be no **copurification** of inhibitors of the RT-step. It **must** be free of nucleases for extended storage. The **most** obvious problem concerns the **degradation** of the RNA and this is best addressed by insisting that every RNA preparation is rigorously assessed for quality. The assessment of RNA **integrity** by inspection of the 28S and 18S ribosomal RNA bands using gel electrophoresis is a **cumbersome**, **low-throughput (low success)**

method and requires **significant** amounts of **precious** RNA.
A second question relates to the **presence of inhibitors** in template RNA preparations. There are **numerous components within blood and tissue that can inhibit** RT-PCR assays. Mammalian blood, especially the heme compound,8 is well known for containing **inhibitors** of the PCR assay,9,10 with as little as **1%** v/v blood inhibiting Taq polymerase.11 **Humic acid** is an **inhibitor** of PCR reactions carried out on samples extracted from soil,12 and inhibitors are present in food,13 with **calcium an important culprit.** 14 One important aspect of any inhibition of the PCR assay is that this may **compromise** PCR as a diagnostic tool. For example, chain-terminating drugs, such as **acyclovir** used in the treatment of **retro** viruses, inhibit Taq DNA polymerase, producing a **false negative** result in some patients.15 **High levels of copurified RNA can also result in failure** of the PCR assay.16 Culture media, components of nucleic extraction **reagents**,13 and **even** the use of **wooden toothpicks** to pick bacterial colonies have been reported as **inhibiting** the PCR reaction.17 Last but not least, **inhibitors** can be selective: **Skeletal** muscle has been reported to contain **inhibitors** that inhibit **one** polymerase—e.g., Taq, but not Thermus thermophilus polymerase.18

Clearly, there is little point in recording spurious differences in mRNA levels that are **based** simply on the

presence of inhibitors in the different templates affecting either the RT or the PCR assay. One way of avoiding this is to test each RNA preparation for **inhibitors** by amplifying an amplicon set that has **no** sequence identity with any known sequence within the target RNA. For example, if one is investigating human gene expression, a plant or artificial amplicons could be used to test each RNA preparation for inhibitors. Practically, this involves preparing a **mastermix** that includes the plant or artificial amplicon, both primers, and the specific probe set. A benchmark Ct (threshold cycle) that is characteristic for that assay in the **absence** of any inhibitor is recorded by adding **water** to that mastermix (the "no added template" control). This acts as a reference point for Ct values obtained when the water is substituted with RNA prepared from cells, biopsies, or body fluids. In the **absence** of inhibitor, the Ct remains the same; in the **presence** of inhibitor, the Ct increases.

It might be thought that the use of a **reference** gene as an endogenous control can **also** identify the presence of inhibitors. This may well be possible for experiments involving RNA extracted from tissue culture cells, although one would have to show that the particular reference gene used is **not** affected by experimental conditions. However, for experiments involving biopsies, the **problem** with this approach is that the mRNA **levels** of reference genes **vary significantly** between different individuals **and** tissues. Without a **priori** knowledge of

mRNA levels in a particular tissue, it is **not** possible to determine whether a particularly low Ct is caused by an inhibitor **or by** low levels of that particular mRNA in that sample. Therefore, we do **not** recommend the use of reference genes for this purpose.

Historically, so-called housekeeping genes, **believed to be** constitutively expressed and **minimally** regulated, have been **used** widely as internal RNA **references** for Northern blotting, RNAse protection, and qualitative RT-PCR analyses. They remain widely used as **reference** genes (**endogenous controls**) for quantitative analysis in real-time RT-PCR assays, usually **without** any real investigation as to **how** invariant , their mRNA levels really are under the **experimental** conditions being investigated. A recent systematic analysis and comparison of their **usefulness** on **in vivo** tissue biopsies has concluded that a single housekeeping gene should **not** be used for normalization.19 It seems reasonable to **assume** that most genes are regulated , and that this will cause significant **unpredictable** differences in their expression patterns **between** , and , **even** within the same individual. If housekeeping genes are to be used, **they must be validated** for the **specific** experimental setup and it is probably **necessary** to choose **more than one**—as was done, for example, for expression profiling of **T** helper cell differentiation.20 The **problems** associated with the selection of appropriate **reference** genes were described

recently in a clear and authoritative manner, wherein the authors recommended using the **geometric mean** of multiple, carefully selected **reference** genes for normalization.21 These authors helpfully provide a **program** that aids in selecting the **most suitable reference genes.**

There are **two** types of homogeneous fluorescent reporting chemistries: **non**specific detection **and** specific detection. Nonspecific detection uses intercalating **dyes** such as SYBR Green that **bind** to any double-stranded DNA **generated?** during the PCR reaction and **emit** enhanced fluorescence. 22 These are simply added as a **reagent** to the PCR **cocktail?** of standard reactions and, **although** intrinsically **non**specific, **can** yield **quasi**template specific data , if DNA **melt?** curves are used to identify **specific** amplification products. 23 Assays using DNA-**binding dyes** have two advantages over probe-based ones: (1) they can be incorporated into optimized and long-established protocols that use legacy primers and experimental conditions, and (2) they are significantly cheaper, as there is no probe-associated cost. This makes them very useful for optimizing a PCR reaction; for example, when testing any interaction between the primers by melt curve analysis, and carrying out initial, exploratory screens of multiple amplicons before using a probe-based protocol. Indeed, despite the nonspecific nature of amplification detection, DNA-binding dye-based assays need **not** be less reliable than

probe-based assays. Interestingly, there is at least one report that suggests that SYBR Green I detection is **more precise and** produces a more linear decay plot than TaqMan detection. 24 **Disadvantages** include their **indiscriminate** binding to any double-stranded DNA, which can **result** in fluorescence readings **in** the "no template controls" (NTC) **due to** dye molecules binding to primer dimers. This can be **minimized** by using separate RT and PCR steps. A **second** problem is that since this assay is **no more specific than conventional** PCR, the use of melt curves is **obligatory**, thus **adding to the complexity of data analysis.** A **third** drawback is that **multiple** dye molecules **bind** to a **single** amplified molecule and consequently the amount of signal generated following irradiation is **dependent** on the mass of double-stranded DNA produced in the reaction. **Assuming** the **same** amplification efficiencies, amplification of a **longer** product will generate **more** signal than a shorter one. If amplification efficiencies are **different**, quantification will be **even more inaccurate.**

Specific Detection

Template-specific analysis requires the design and synthesis of one **or more** custom-made fluorescent probes for **each** PCR assay. Most reporting systems utilize fluorescent resonance energy transfer (FRET) or similar interactions between donor and quencher molecules as the **basis** of detection. The types of reporters used for these

probes include **fluorescein, rhodamine, and cyanine dyes, and derivatives thereof; some also have** either fluorescent or nonfluorescent acceptors on the same or on a complementary molecule. There is a **huge** selection of fluorescent dyes, mainly because the chemistries for label incorporation into nucleic acid probes , are well developed since they are used in **other** molecular biology procedures such as DNA sequencing. All chemistries follow the **same** principle: A fluorescent signal is **only** generated **if** the amplicon-specific probe **hybridizes** to its complementary target. In addition, some probes **may also** be used in melt-point analyses to provide **additional** identification of amplified product. The main advantage of specific chemistries is that specificity **no longer** resides in the primers; instead, the use of a probe **introduces? an additional level of specificity.**

Nonspecific amplification due to **mis**priming or primer–dimer artifacts does not generate a signal and is **ignored?** by the fluorescence detector. This obviates?? the need for post-PCR Southern blotting, sequence analysis, or melt curves to confirm the identity of the amplicon. Another advantage over **intercalating** dyes is that the probes can be labeled with different, distinguishable **reporter dyes** that allow? the detection of amplification **products?** from ‘several distinct sequences’? in a single PCR reaction (multiplex). However, the **absence** of detection is **not** the **same** as the **absence** of **artifacts**, and **non**specific amplification can, and indeed does, **affect** amplification

efficiency and any subsequent quantification. The **major disadvantage** is that because of its specificity, **artifacts that interfere with amplification efficiency cannot be detected.** Therefore, **intercalating dyes** should be used to optimize primers and reaction conditions **prior** to any quantification experiments to ensure the absence of amplification **artifacts**. Another disadvantage is the cost associated with these chemistries: Each target requires its own specific probe. This becomes particularly painful when quantifying multiple targets, as costs escalate very rapidly.

One of the key advantages of real-time PCR assays is their **wide dynamic range,** which allows the researcher to compare Ct values obtained from samples containing **hugely different** levels of DNA. The difference between a PCR assay **and** an RT-PCR assay is that the latter reaction can be initiated in **three** different ways, which of course has the potential to **result in variable results.** cDNA **priming** can be carried out using **random** primers, oligo-dT, **or** target-specific primers. Each of the three methods **differ significantly** with respect to cDNA yield and variety as well as specificity and, since the choice of **primer** can **cause marked variation** in calculated mRNA copy numbers, 25 the implications of using any particular method should be considered carefully. 26 It is worth pointing out that the **melting temperature** of both random primers and oligo-dT is **well below** the optimum temperature of thermostable RTs; hence, **neither** can be

used with thermostable RT **enzymes without some low-temperature preincubation step or primer modification** (e.g., locked nucleic acid substitution of a nucleotide27). Ambion have shown that **un**intended endogenous priming can occur **regardless** of , which primers are used to prime the RT reaction. Using 32P-labeled **avian myeloblastosis virus** (AMV), Moloney murine leukemia **virus** (MMLV), and RNaseH−MMLV **reverse transcriptases,** they performed standard RT reactions with **and** without primers. They found that the resulting products were **identical**, and concluded that the cDNA **generated** in the RT reactions was the **result** of endogenous random priming (http://www.ambion.com/catalog/CatNum.php?1740 RNA Purification by Invitrogen https://www.thermofisher.com/in/en/home/brands/invitrogen/ambion.html).

Such **non**specific priming can lead to **lowered and/or variable** signal in the subsequent PCR assay, although **how much** of a problem this is, in real life, remains **unclear**. Not surprisingly, Ambion's EndoFree RT kit addresses this problem. Random primers prime RT at multiple points along the transcript, hence producing **more than one** cDNA transcript per original target. Thus this method is by definition **non**specific, **but yields the most** cDNA and is **most** useful for transcripts with significant **secondary** structure. First-strand cDNA

synthesis with random primers should be conducted at **room** temperature. However, the majority of cDNA synthesized from total RNA **will be** ribosomal RNA-derived. This could **create real problems** if the target of interest is present **at low** levels, as it **may not** be primed effectively by random primers and its amplification **may not** be quantitative. Indeed, it has been demonstrated that random hexamers can **overestimate** mRNA copy numbers by **up to 19-fold** , compared with a sequence-**specific** primer. 25 It has been described **as the least reliable method of priming** cDNA26; nevertheless, as with any experimental protocol, this random priming of cDNA can yield reliable and reproducible results**??? if it is carried out** in a careful, competent manner.
One added advantage of random priming is that it **generates the least bias in** the resulting cDNA. 6 cDNA synthesis using oligo-dT **is more specific to** mRNA than random priming, as it will **not** transcribe rRNA. It can **struggle** to generate transcripts from mRNAs with significant **secondary** structure, and obviously it will **not** prime any RNAs that **lack** a polyA tail, e.g., those specifying histones or viral RNAs. However, since oligo-dT priming requires **very high-quality** RNA that is **full length,** it is **not** a good choice for transcribing RNA that is likely to be **fragmented**, such as that typically obtained from **laser** capture microdissected tissue or from **archival** material. Furthermore, the RT may **fail** to reach the primer probe binding site **if** secondary structures exist that

impede its processivity or **if** the primer/probe binding site is at the extreme 5′-end of a **long** mRNA. This may be the case **if** the mRNA contains a very long untranslated 3′-region or **if** splice variants differ at the 5′-end of the mRNA (e.g., the MHC class II transactivator isoforms I, III, and IV). Target-specific primers synthesize the most specific cDNA and, all things being equal are **probably** the **most sensitive** option for quantification. 26 The main **disadvantage** of this method is that it requires **separate** priming reactions for **each** target; hence it is **not** possible to **return** to the **same** preparation **and** amplify other targets at a later stage. It is also **wasteful** if only limited amounts of RNA are available. In our experience, the use of target-specific oligonucleotides to prime cDNA gives superior results to using random primers. In particular, we find that a reaction primed by target-specific primers is linear over a **wider** range than a similar reaction primed by random primers.

However, there does **appear to be gene-specific variation and, as always, it is** important to validate individual assays **using standard curve** dilutions before coming to **conclusions** about results obtained from actual samples. As always, dogma is the enemy of progress, and a properly validated, executed, analyzed, and interpreted real-time RT-PCR assay carried out using random primers is infinitely **preferable** to a poorly designed, hastily executed, **inappropriately** analyzed and gene-specific primed assay.

The resolving power of RT-PCR is **also limited** by the efficiency of RNA-to-cDNA conversion, **which depends on the enzyme used.** However, the conversion efficiency is significantly (greater than 3-fold) **lower** when target templates are **rare** and it is **negatively** affected by **non**specific or **background** RNA present in the RT reaction. 28 Of course, considerations of linearity of the RT step are **just** one side of the equation. Another consideration concerns the **"Monte Carlo"** effect, an **inherent limitation of** PCR amplification from small amounts of any complex template due to differences in amplification efficiency between individual templates in an amplifying cDNA population. 29 Every template has a certain probability of being amplified **or being lost and, once diluted past a certain threshold, copy number will display large variations in amplification.** The Monte Carlo effect is **dependent** upon template concentration: The **lower** the abundance of any template, the **less likely its** true abundance will be reflected in the amplified product. One model for this phenomenon considers primer annealing to any individual template molecule during **each** PCR cycle as a random event. Under conditions of primer **excess**, the probability of primer annealing is **dependent** upon annealing temperature, annealing time, and the number of available templates. If the **number** of molecules of a particular template is **limiting**, then that template within a complex

mixture will have slight and random **differences** in amplification efficiencies **depending** upon whether the primers were able to anneal. If these differences occur early in the PCR assay, **large variations in final product concentration can be produced during the exponential phase of the amplification reaction.** cDNAs of **lower** abundance will be more likely to experience the **Monte Carlo** effect, since their **probability** of primer annealing is lower. Unfortunately, **this situation is difficult to resolve, since many experiments are designed to identify very low target mRNAs.**

One solution is to use mRNA, rather than total RNA preparations. This may improve primer-binding efficiency, as it would **reduce?** significantly the complexity and quantity of unrelated template present during primer/target annealing. However, **preparation of mRNA involves additional steps, may lead to the loss of some mRNA, and it is more difficult to assess the quality of the final product.**

Nevertheless, **if ultimate sensitivity** is the main consideration, the use of mRNA may be advisable. In addition, all assays quantitating **very low** target copy numbers should be run **in triplicate and be repeated at least once,** so that any problems with reproducibility become immediately apparent. Nevertheless, it is worth emphasising that real-time RT-PCR, like any other assay, **will not generate quantitative results at the limits of its**

sensitivity. One of the major advantages of including a standard **curve** with **every** run is that its **highest** dilutions provide an immediate benchmark for the assessment of the quality of the results obtained from the **unknown** samples. The **highest** dilution of the standard curve to report consistently concordant Ct values **delineates** the lowest copy number that can be quantitated with confidence. If the Ct values recorded by any **unknowns** translate into copy numbers **lower** than that benchmark, they should be recorded **as qualitative** (yes/no) results. The Ct has **become the parameter** most conveniently and most frequently quoted when reporting qRT-PCR results. However, it is important to consider carefully **what** the Ct actually **reveals** , and to ask whether quoting a Ct is sufficiently informative , to allow a confident assessment of any conclusion drawn **from** a real-time RT-PCR experiment. The threshold cycle (Ct) is **defined** as the cycle when sample fluorescence **exceeds** a chosen threshold above calculated background fluorescence. The **critical word is "chosen,"** since background fluorescence is **not** a constant or absolute value **but** is influenced by changing reaction conditions. Hence, **if** background fluorescence varies, the value of a Ct recorded for any particular sample **is also going to be variable.** Since the Ct is **central** to an appropriate understanding of the real-time assay, and at the same time is frequently **mis**understood, it is **important** to spell out the parameters governing its value. The Ct is at the **heart** of the qRT-

PCR assay, as it is used to determine copy **numbers**, which is of course the **whole** point of carrying out a quantitative assay. **A positive Ct (defined as a fluorescence reading of less than the final cycle number) can arise , due to genuine amplification, but some Ct values are not due to genuine amplification and some genuine amplification does not record a Ct.** One **important reason** for a real amplification **not** recording a Ct is the wandering (drifting) baseline **caused** by an **incorrectly set** background cycle range. This range specifies the cycles that will be used to **calculate** the threshold fluorescence levels. Typically, it encompasses **only early** PCR cycles **prior** to the accumulation of significant amplification products, e.g., 3 through 15 on the Applied Biosystems PRISM 7700 or 5 through 9 on the Stratagene instruments. The **background** signal in **all wells** is used to determine the "baseline fluorescence" across the entire reaction plate. However, sometimes this does **not generate** an accurate background reading for that individual well. A comparison of two amplification plots shows that they have very similar ΔRn values (baseline-corrected normalized fluorescence) (0.023 vs. 0.02), but whereas one evidently crosses the default threshold, the other one remains well below it (see Fig. 55).). This is **because** the fluorescence levels in the green well remain fairly constant throughout the early stages of the PCR assay, and start rising from approximately the same level

recorded at the end of the baseline cycle. The fluorescence represented by the **red** line, on the other hand, drifts downwards significantly by 0.015 units, and the rise recorded following probe hydrolysis is **not sufficient** to allow the amplification plot to cross the default threshold.

It is clear from the amplification plots that , it is **not** correct to report one well as recording a positive Ct and the other well a negative one. This is where appropriate baseline **correction** comes in: An adjustment of the baseline cycles to include the lowest point of the amplification plot corrects for this fluorescence drift and allows this well to record a correct Ct that is very similar to the one recorded by the **green** well.

The most valuable application of such baseline corrections is when analyzing negative controls and detecting clear evidence of amplification, which is too low to cross the default threshold level. Indeed, if these corrections result in a negative control becoming positive, this becomes the **critical** component of the analysis.

Many instruments now provide the choice of a default adaptive baseline enhancement, which **automatically** calculates the best baseline for each plot individually, thereby providing the **most accurate Ct.**??

The threshold **calculated** by the real-time instrument **depends** on the baseline, and the **default** settings are usually **not** altered in standard runs. However, they may need to be **changed** if specific conditions arise, usually

linked to the high Ct and low ΔRn values associated with very low target copy numbers. Two points are worth noting: There need **not** be a single threshold for each run. For example, Applied Biosystems acknowledge that data from a **single** run can be **analyzed** with multiple threshold values and they refer to a "window or range of values within which a threshold setting will fit."30 Indeed, the thresholds **calculated** by other instruments (e.g., those from Stratagene and the latest Applied Biosystems instruments) **vary depending on** what well or combination of wells are being analyzed. The threshold must intersect the **exponential** phase of individual amplification plots. **If** the Ct values are **very high and** the ΔRn values are very low, there may well **not** be a clearly defined exponential phase of the amplification plot. In such cases it will be necessary to make threshold **adjustments** that generate a (qua**li**tative) positive sample, with actual qua**nt**ification quite irrelevant, as it would **only** generate an **in**accurate copy number. However, it is also clear from experience that **multiple** thresholds are the **exception** rather than the rule for the vast majority of runs that target, medium-level mRNAs. Nevertheless, sometimes **multiple** thresholds are **the only way** that the data can be analyzed fairly; this is of **particular** importance when **negative** controls are involved. One **example** of when to use multiple thresholds is **when** there are **clear signs** of amplification in a negative control, and

application of the default baseline and/or threshold would **result in a negative** Ct.

Altering the threshold, or the baseline if a wandering baseline is the problem, usually corrects this technical inconsistency and allows the operator to record a positive Ct.

Of course, the whole question of **how to interpret a positive** NTC is **the subject of many a heated debate.** Interestingly, **no guidelines have been published on this matter.** Therefore, the proposals below are based on our views and are **not** meant to be definitive. We acknowledge that the cut-off points are **arbitrary**, but they represent a common-sense approach and a **starting point for a discussion of this subject that needs to be carried out.** In some instances, the situation is quite clear: If all unknown samples record C_t values of around, say 18–25, and the NTC records a C_t of 39, then it is legitimate to ignore the very high C_t values recorded for the NTC and use the data. However, we recommend that the fact that the NTC did record a positive C_t be noted in the results section of any publication reporting those data. The **only exception** to this rule would be an NTC recording a C_t less than 30, for this **suggests the presence of high levels of contamination somewhere in the laboratory and assay set-up and** requires urgent attention.

The situation is quite different if the values for **unknown** sample and NTC C_t are more comparable. Again, this is usually an issue **only** when quantitating RNA from single cells or from laser capture microdissected tissue, but it is a crucial one since "Caesar's wife must be above suspicion." We suggest that any C_t that differs by more than 5 from the NTC **be regarded as probably not caused by any contaminant,** especially when the replicate wells also record positive, similar C_t values. Of course, **if one replicate** records a positive C_t, and the other(s) is negative, then that sample must be treated with **the utmost suspicion and** certainly can **never** be called a positive. At the very least, the sample must be **rerun**, ideally using **more** template, and generate its positive C_t in the **absence** of any NTC contamination.
If the ΔC_t separating the unknown sample from the NTC is greater than 5 C_t, that sample is as likely to have become **contaminated** as **not** and must be rerun, again using more template RNA. The rationale behind using more RNA is that for every doubling of input template, the C_t should increase by about 1. For example, if the unknown recorded a C_t of 37.5 and the NTC a C_t of 39.0, then the only acceptable result, in our opinion, would be a **new** C_t of around 36.5, if the amount of template RNA had been **doubled**. Of course, the NTC would have to record a C_t of 45. The NTC is such a crucial part of a good experimental setup that the requirement for an **absolutely** negative result cannot ever be compromised.

Therefore, we **propose** that if the C_t values recorded by unknowns are above 33–35, then the NTC must always be negative for any results to be valid. Finally, if the unknowns record C_t values in the region of 37–39, it is important to run the reaction for 45 cycles, to be certain that the NTC comes up negative. Clearly, if an unknown records a C_t of 39.5, and the run ends after 40 cycles, any NTC that would have recorded a C_t of 40.01 would come up as a negative. Additional advice would be to try and **redesign** the assay to make it as efficient as possible, thus lowering the cycle number when the instrument first detects amplification product. The whole question of **amplification efficiency** is very well discussed elsewhere.[31,32]

Incidentally, the question of where to place the NTC and how many NTCs are required per well is also worth a brief mention. In our **opinion**, in a 96- or 384-well assay, NTCs should always be run in the row below and next to the lowest dilution of the standards. There should be at least two NTC controls with triplicate replicates. One NTC should be sealed prior to the addition of any unknowns, positive controls, or standard templates. The second NTC should be sealed after the addition of any unknowns, positive controls, or standard templates.

The reporting of C_t values **alone can conceal as much as it reports,** we believe it is necessary to begin a concerted effort to introduce **more standard analysis and**

reporting procedures. There is **the absence of such standards for** real-time RT-PCR.
Of course, choice of chemistries, primers and probes, and instruments must be appropriate to whatever is being quantitated.

Finally, data must be **interpreted !!!!!!**, and **this remains a real problem.**
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2291693/
data must be **interpreted !!!!!!**

Potential False-Positive Rate Among the 'Asymptomatic Infected Individuals' in Close Contacts of COVID-19 Patients G H Zhuang 1 , M W Shen, L X Zeng, B B Mi, F Y Chen, W J Liu, L L Pei, X Qi, C Li
Abstract .. Chinese Objective: As the prevention and control of COVID-19continues to advance, the active nucleic acid test screening in the close contacts of the patients has been carrying out in many parts of China. However, the false-positive rate of positive results in the screening has not been reported up to now. But to clearify the false-positive rate during screening is important in COVID-19 control and prevention. Methods: Point values and reasonable ranges of the indicators which impact the false-positive rate of positive results were estimated based on the information available to us at present. The false-positive rate of positive results in the active screening was

deduced??, and univariate and multivariate-**probabilistic** sensitivity analyses were performed to understand the robustness of the findings. Results: When the infection rate of the close contacts and the sensitivity and specificity of reported results were taken as the point estimates, the positive predictive value of the active screening was only 19.67%, in contrast, **the false-positive rate of positive results was 80.33%.** The multivariate-**probabilistic** sensitivity analysis results supported the base-case findings, with a 75% **probability** for the false-positive rate of positive results over 47%. **Conclusions**: In the close contacts of COVID-19 patients, **nearly half or even more** of the 'asymptomatic infected individuals' reported in the **active nucleic acid test** screening might be false positives.
https://pubmed.ncbi.nlm.nih.gov/32133832/

Why have so many coronavirus patients died in Italy? The country's high death toll is due to an **aging** population, **overstretched** health system and **the way fatalities are reported**. By Sarah Newey, 22 March 2020 •
The coronavirus pandemic is exacting a heavy toll on Italy, with hospitals overwhelmed and a nationwide lockdown imposed. But experts are also concerned about a seemingly high death rate, with the number of fatalities outstripping the total reported in China. Of the 47,000 people confirmed coronavirus patients in Italy, 4,032 so far have died - with **a record increase** of 627 in the last

24 hours. By contrast **China has almost twice as many cases,** 81,250, but 3,253 fatalities. In **very crude** terms, this means that around **8%** of confirmed coronavirus patients have died in **Italy**, compared to **4%** in **China**. By this measure **Germany**, which has so far identified 13,000 cases and 42 deaths, has **a fatality rate of just 0.3**% . **So why the disparity?** According to Prof **Walter Ricciardi,** scientific adviser to **Italy's** minister of health, the country's mortality rate is **far higher due to demographics** - the nation has the **second oldest population worldwide** - and **the manner in which hospitals record deaths.** "The **age** of our patients in hospitals is substantially **older** - the median is 67, while in **China** it was 46," Prof Ricciardi says.
"So essentially the **age** distribution of our patients is squeezed to an **older** age and this is substantial in increasing the lethality." A study in JAMA this week found that almost **40%** of infections and **87%** of deaths in the country have been in patients **over 70 years old.** And according to **modeling** the majority of this age group are likely to need **critical** hospital care - including **80%** of 80-somethings - putting **immense** pressure on the health system. But Prof Ricciardi added that **Italy's death rate may also appear high because of how doctors record fatalities. "The way in which we code deaths in our country is very generous in the sense that** all the people who die in hospitals **with** the coronavirus are deemed to be dying **of** the coronavirus. "On re-evaluation by the

National Institute of Health, **only 12**% of death certificates have shown a **direct??** causality from coronavirus, while **88%** of patients who have died **have at least one pre-morbidity - many had two or three,"** he says. This does not mean**???** that Covid-19 did not contribute to a patient's death, rather it demonstrates that Italy's fatality toll has surged as a large proportion of patients have underlying health conditions. Experts have also **warned against** making **direct comparisons between countries due to discrepancies in testing.**

Martin McKee, professor of European public health at the London School of Hygiene and Tropical Medicine, says that **countries do not yet have a good indication of how many mild** infections they have. If further testing finds more asymptomatic cases spreading undetected, the **mortality rate will drop.**
"It's too early to make a comparison across Europe," he says. "We do not have detailed sero-surveillance of the population and we do not know how many asymptomatic people are spreading it." Prof McKee adds that **testing is not currently consistent across the continent, or world.** "In **Germany**, epidemiological surveillance is more **challenging** - simply **because** of the **complexity** of working in a federal state and **because** public health is organised very much at the **local** level." But there are **other** factors that may have contributed to **Italy's** fatality rates, experts say. This includes a **high rate of smoking**

and pollution - the majority of deaths have been in the northern region Lombardy region, which is **notorious for poor air quality.** Workers stand next to coffins and remains of the coronavirus?? victims, in Bergamo, Italy. And there's also **no** question that parts of Italy's health system have been **overwhelmed** with a surge of coronavirus patients and are **struggling** to cope. "Doctors in Italy haven't been dealing with one or two patients in care... but up to 1,200," says Dr **Mike Ryan,** health emergencies programme executive director at the World Health Organization. "The fact they're saving so many is a small miracle in itself." This pressure is likely to get worse as more healthcare workers are infected and have to isolate - already, 2,000 have contracted the virus**??** in Italy. "There are **three** factors involved in Italy: **one** is that it is a **much older** population, **two** the health system was overwhelmed, and **three** there has been a significant loss of health workers because of a high coronavirus infection rate among them," says Prof McKee. "Italy is ahead of us in the epidemic - and it's not clear how many health workers [in the UK] are having to self isolate. That's another big concern. "Based on Italy's experience, there is a real concern for the UK," adds Prof McKee. "Compared to almost every other European country we have a relative shortage of ventilators and medical staff." https://www.telegraph.co.uk/global-health/science-and-disease/have-many-coronavirus-patients-died-italy

Is the 2019 Coronavirus **Really a Pandemic?**
David Crowe..March 21, 2020
http://theinfectiousmyth.com/book/CoronavirusPanic.pdf
False positives: Chinese paper documenting 80% false positive rate.
Introduction The Coronavirus scare that emanated from Wuhan, China in December of 2019 **is an epidemic of testing. There is no proof that a virus is being detected by the test and there is absolutely no concern about whether there are a significant number of false positives on the test.**

What is being published in medical journals is not science, every paper has the goal of enhancing the panic by **interpreting** the data **only in ways that benefit the viral theory, even when the data is confusing or contradictory.** In other words, the medical papers are **propaganda**. It is also an epidemic by **definition**. The definition, which **assumes** perfection from the test, **does not have the safety valve** that the definition of SARS did, thus **the scare can go on until public health officials change the definition or realize that the test is not reliable.** 1 Officially the virus? is called SARS-CoV-2 and the disease it is believed to cause, COVID-19. We will just refer to coronavirus for the current virus panic, **and** SARS for the **2003** panic. 2 What I learned from studying SARS, the **previous** big coronavirus scare,

after the 2003 epidemic, was that **nobody had proved a coronavirus existed, let alone was pathogenic.** There was evidence **against** transmission, and afterwards, negative assessments of the extreme treatments that patients were subjected to, the nucleoside analog antiviral drug **Ribavirin**, **high dose corticosteroids, invasive respiratory assistance, and sometimes oseltamivir (Tamiflu).** This is documented in: http://theinfectiousmyth.com/book/SARS.pdf
3. Executive Summary The world is suffering from a **massive delusion based on the belief that a test for RNA?? is a test for a deadly new virus,** a virus that has emerged from wild bats or other animals in China, **supported by the western assumption that Chinese people will eat anything that moves.** If the virus exists, then it should be possible to purify viral **particles**. From these particles RNA can be extracted **and** should match the RNA used in this test. **Until** this is done it is possible that the RNA comes from **another** source, which could be the cells of the patient, bacteria, fungi etc. There might be an association with **elevated** levels of this RNA and illness, **but that is not proof that the RNA is from a virus. Without** purification and characterization of virus particles, it **cannot** be accepted that an RNA test **is proof that a virus is present. Definitions** of important diseases are surprisingly **loose**, perhaps embarrassingly so. A couple of symptoms, maybe contact with a previous patient, and a test of **unknown** accuracy, is all you often

need. While the **definition** of SARS, an earlier coronavirus panic, was self-limiting, the **definition** of the **new** coronavirus disease is **open-ended, allowing the imaginary epidemic to grow.** Putting aside the existence of the virus, if the coronavirus test has a problem with **false** positives (**as all** biological tests do) then testing an **un**infected population will produce **only** false-positive tests, and the **definition of the disease will allow the epidemic to go on forever.** This **strange** new disease, officially named COVID-19, has **none** of its own symptoms. Fever and cough, previously blamed on uncountable viruses and bacteria, as well as environmental contaminants, are most common, as well as abnormal lung **images**, **despite** those being found in healthy people. Yet, **despite** the fact that **only a minority** of people tested will test positive (often less than 5%), it is **assumed** that this disease is easily recognized. If that was the truly the case, the majority of people selected for testing by doctors should be positive. The coronavirus test is based on **PCR**, a DNA **manufacturing** technique.

When used as a test , it does not produce a positive/ negative – binary result, but simply the number of cycles required to detect sufficient material to beat the arbitrary cutoff between positive and negative.

If positive means infected and negative means uninfected, then there are cases of people going from infected to uninfected and back to infected again in a couple of days.

But once people test positive, they are likely to be treated, with treatments similar to SARS. Doctors faced with what they **believe** is a deadly virus treat for the **future**, for anticipated symptoms, not for what they see today. This leads to the use of **invasive** oxygenation, high dose corticosteroids, 'antiviral' drugs and more. In this case, some populations **of** those diagnosed (e.g. in China) are **older and sicker than** the general population and **much less able to withstand aggressive treatment.**
After the SARS panic had subsided , doctors reviewed the evidence, and it showed that **these treatments were often ineffective, and all had serious side effects, such as persistent neurologic deficit, joint replacements, scarring, pain and liver disease.** As well as **higher mortality.**

4. **Virus Existence**
Scientists are detecting novel RNA in multiple patients with influenza or pneumonia-like conditions, and are **assuming** that the detection of RNA (which is believed to be wrapped in proteins **to form an RNA virus,** as coronaviruses are believed to be) is **equivalent to isolation of the virus. It is not,** and one of the groups of scientists **was honest enough to admit this:** "we did

not perform tests for detecting infectious **virus** in blood" [2] But, **despite** this admission, earlier in the paper they repeatedly referred to the 41 cases (out of 59 similar cases) that "tested positive for this RNA" as, "41 patients… **confirmed** to be infected with 2019-nCoV." Another paper quietly admitted that: **"our study does not fulfill Koch's postulates"** [1] Koch's postulates, first stated by the great German bacteriologist Robert Koch in the late 1800s, can simply be stated as: • Purify the pathogen (e.g. virus) from many cases with a particular illness. • Expose **susceptible** animals (obviously **not** humans) to the pathogen. • Verify that the **same** illness is produced. • Some add that you should **also re-purify** the pathogen, just to be sure that it really is **creating** the illness.

Famous virologist **Thomas Rivers** stated in a 1936 speech, "It is obvious that Koch's postulates have not been satisfied in viral diseases". That was a long time ago, but the problem **continues**. **None** of the papers referenced in this article have even attempted to **purify** the virus. And the word '**isolation'** has been so debased by virologists , **it means nothing (e.g. adding impure materials to a cell culture and seeing cell death is 'isolation').** Reference [1] did publish **electron** micrographs, but it can clearly be seen in the lesser magnified photo, that the particles **believed to be** coronavirus are **not** purified, as the **quantity of material**

that is cellular is much greater. The paper notes that the photos are from "human airway epithelial cells". Also consider that the 4 photo included in the article will certainly be the "**best**" photo, **i.e. the one with the greatest number of particles.**

Lab technicians may be encouraged to spend hours to look around to find the **most** photogenic image, the one that **most** looks like pure virus. There is **no way to tell that** the RNA being **used** in the new coronavirus PCR test is found **in those particles seen in** the electron micrograph. There is **no** connection between the test, and the particles, and **no** proof that the particles are viral. A **similar** situation was revealed in March 1997 concerning HIV, when **two** papers published in the **same** issue of the journal "Virology" revealed that the **vast majority of what had previously been called "pure HIV" was impurities that were clearly not HIV, and the mixture also included micro-vesicles that look very similar to HIV under an electron microscope, but are of cellular origin.** [5][6]

5. Disease Definition

Infectious diseases **always** have a **definition**, but they are usually **not publicized too widely because then they would be open to ridicule.** They usually have a **"suspect case"** category based on symptoms and exposure, and a "**confirmed**" category that adds some

kind of testing. Reference[13]describes a **suspect case definition** for the novel coronavirus, derived from WHO **definitions** for SARS and MERS (Middle East Respiratory Syndrome). This **definition** was in effect **until** January 18, 2020, and **required all four of** the following criteria: • "fever, with or without recorded temperature". Note that there is **no universal definition of fever,** so this may just be the **opinion** of a physician or nurse. With SARS a **fever** was **defined** as 38C even though **normal** body temperature is considered to be 37C (98.6F). • "**radiographic** evidence of pneumonia". This can occur without illness, as was seen in [3] – a 10 year old boy with **no** clinical symptoms. He was diagnosed with pneumonia **despite** this. • "**low or normal** white-cell count or low lymphocyte count". This is **not** really a criterion as every healthy person is included. This is also **strange** because people suffering from an infection **normally** have **elevated** white blood cell counts (although they may drop in people **dying** from an infection). • One of the following **three**: 1. "no reduction in symptoms after antimicrobial treatment for 3 days". This is a standard indication of a 'viral' pneumonia, i.e. one that does **not** resolve with antibiotics. 2. "epidemiologic link to the Huanan Seafood Wholesale Market". This, and the **next** criterion, **create the illusion** of an **infectious** disease, as it prefers the diagnosis of connected cases. 3. "contact with other patients with similar symptoms".

On January 18th the last, **three**-part category was **changed** to: **five** • One of the following: 1. “travel history to Wuhan” 2. “direct contact with patients from Wuhan who had fever or respiratory symptoms, within 14 days before illness onset” The **big** problem is that, in contrast to the definition for SARS, **a “confirmed case” did not originally require the criteria for a suspect case to be met.** A “confirmed case” **simply** required a positive RNA test, **without** any symptoms or possibility of contact with previous cases, illustrating **total faith** in the PCR technology used in the test.

The World Health Organization **definition** [15] has the **same flaw.** It was the fact that the SARS definition required **both** a reasonable possibility of contact with a previous case, **and** symptoms, that allowed the epidemic to burn out. Once everyone was quarantined, contact with an existing case was highly **unlikely**, testing stopped, and doctors could declare victory. The **Chinese** eventually woke up and, around February 16th **required confirmed cases to meet the requirements for a suspected case, as well as a positive test.** They may have put this **new definition** into practice **earlier** because after a massive addition of almost 16,000 confirmed cases on February 12th, the number **fell dramatically each day and,** by February 18th was under 500 cases, and continued to stay low. But **other** countries did **not**

learn**??**. Korea, Japan and **Italy** (and perhaps **other** countries) have started doing tests on people with **no** epidemiological link, encouraging people with the **vague** symptoms that are part of the **definition** to come to hospital to get checked, and **obviously** following up with anybody with a connection to them, most of whom will be asymptomatic. **Consequently**, in mid to late February, cases in those and other countries **started to skyrocket.**

A New Disease? COVID-19, to use its formal name, is described as a **distinct new** disease. But it clearly is **not**. There are **no** distinctive symptoms, for a **start**. Reference [2] showed that, among 41 early cases, **the only** symptoms found **in** more than half, were fever (98%) and cough (76%). 98% had CT Scan imaging showing problems in both lungs (although it is possible to have shadowing on a CT scan **without** symptoms). The high percentage of cases with fever and shadowing in both lungs is an **artefact** of the disease **definition**, fever and "radiographic evidence of pneumonia" are two of the diagnostic criteria for a **probable** case. The **low rate** of people testing **positive** on the coronavirus test is further evidence that there are **no** obvious symptoms. If there were recognizable symptoms, doctors should have a better than 3-5% chance of guessing who has the virus. While some of the people may have been tested, without symptoms, because they were on a flight or cruise**,** countries **outside** China are encouraging people with the

non-specific symptoms of fever and cough to get tested, so increasingly people have symptoms of the **flu or pneumonia, but** are still testing negative in high numbers. 6 For example, as of March 9th, Korea had found 7,382 positive cases out of 179,160 people tested (4.1%) [20]. **In Washington State, where they appear to be reluctant to test anyone,** only 1 out of 27 tested by February 24th had tested positive (**3.7%**) [21]. Perhaps if they had tested all 438 who were then under quarantine, the epidemic **would have exploded from** 1 to about 16 cases (**3.7%** of 438). By March 9th, 1,246 tests had been performed in **Washington** with 136 found positive (**11%**). Obviously, **in neither location can doctors recognize cases clinically.**

6. Testing

Assuming, for a moment, the **existence** of a **new** coronavirus,

what would a coronavirus test tell us, at this stage? Or rather, what does it **not** tell us? • **Without** purification and exposing animals to viral particles we do **not** know **if** the virus is pathogenic (disease causing). It could be an opportunistic infection (invades unhealthy people with weakened immune systems) or a **passenger** virus (that is carried along by risky behavior, such as eating an animal carrier of a virus?). • We **don't** know the **false** positive rate of the test without widespread testing of healthy

people **far** from places where people are being diagnosed with this possible new disease. **If** the test is **99**% accurate, in a city of over 10 million, like Wuhan, there would be about 100,000 false positives (1%). **It is easy to generate a false epidemic if you just keep testing like this.** And it is **worse** if you **restrict** the test to people with symptoms, because then the **flaws** in the test will **not** be revealed for longer. • If someone is sick there is **no** proof that any or all of their symptoms are **due to** the virus, **even if it is present.** Some people may be immune, some may have some symptoms caused by the virus, **but others caused by the drugs they are given, by pre-existing** health conditions, and so on. • We don't know if the people who test **negative** are infected or not, especially when they show up with **similar** symptoms. For example, in [2], out of 59 patients, only 41 tested positive, but the researchers were clearly **not** sure whether the remaining 18 were uninfected or **not**. If they truly are **not** infected, they lend weight to the coronavirus **not** being the cause of their illness, as they **had** symptoms **indisti**nguishable from the 41 positives. Testing at such **an early stage** of knowledge is incredibly dangerous. It spreads panic, it can put people on **dangerous** medications, other circumstances of their **treatment** can be physically and psychologically damaging (such as intubation and isolation, and even seeing all the doctors and nurses in special suits emphasizing how deathly sick you are).

False Negatives – Big Problem
According to an article in the South China Morning Post [23], Li Yan, head of the diagnostic center at the People's Hospital of Wuhan University, noted on Chinese state TV that **because of the multi-step process, an error at any stage could result in an incorrect outcome,** and Wang Chen, president of the Chinese Academy of Medical Sciences, also on CCTV, said the **accuracy is only 30 to 50 percent.**

Wang Chen really means, however, that the test **only** ever produces false negatives, and **never** false positives.!!!!!

In a paper documenting a cluster of illness and positives tests in a family [3], this **bias** is clear, as **most** patients had **more negative** tests than positive tests, **but** were considered positive anyway. Patient 1 had 3/11 positive (27%), patient 2 had 5/11 (45%), patient 3 had all 18 negative, patient 4 had 4/14 (29%), patient 5 had 4/17 (24%) and patient 7 was the only with a majority positive (64%). The only way to decide logically and scientifically **is to have a gold standard for presence of the virus, which can only be** purification and characterization (identification of the RNA and proteins). Since this has **never** been accomplished, doctors get to make decisions on the fly, **biased** towards treating patients **as infected.**

False Positives – Best Evidence

The **first major** attempt to **define** the false positive rate was in a paper describing a **new** test methodology, but it has a **built-in conflict of interest** [19]. Clearly, **if the false positive rate was high,** the authors' aim to "develop and **deploy** robust diagnostic methodology for use in public health laboratory settings", would have **failed**. They did, however, do more than most. They took **297** samples of **nasal** and **throat** secretions **from** biobanks and tested them, only finding "**weak** initial reactivity" in **four** samples which, upon **re**testing, **dis**appeared. The **problem** with this kind of analysis is that biobank samples may **not** have been obtained in the same way as samples from **live** people in an epidemic panic. The sampling was also **not blinded**, something that is necessary to eliminate **the possibility of unconscious bias** (a real problem in medicine). Furthermore, many samples in people believed to be **infected are negative,** and multiple samples are tested, as described for the family cluster paper. In sum, testing 297 samples could, at best, show that the **false positive** rate was 1/300, but **because** multiple samples are often taken, with any one positive sample over-ruling all the negatives, the false positive rate could be considerably **less**, as the **biobank samples were only tested once.** And, even if this test did have a false positive rate that was **very low,** it is **not** clear that this particular test is in use, and the false positive rate can**not** be extrapolated to any other test

design. Even a **small** false positive rate is **critically** important. A 99% accurate test would produce 100,000 false positives in a city of 10 million, like Wuhan. And if the number of positives in sampling is around 4% (which it appears to be from early statistics), then 1 out of 4 positives would be false.

8 Finally, on March 5th 2020 some **Chinese** scientists dropped a bombshell. According to their analysis, "the false-positive rate of positive results was **80.33%".**[26] As the English translation is slightly stilted: **80% of the positive tests did not indicate an infected person.**

Positive, Negative, Positive Again – Confusion.
Some people have fully recovered from illness **blamed** on coronavirus, started to test negative, and then tested positive again. According to a news report [22] patients are **not** considered cured in China until they no longer have symptoms, have clear lungs, and have two negative coronavirus tests. Despite this, 14% of discharged patients later tested positive, but with no relapse of symptoms. This is very difficult to explain **if the test is for a virus,** much easier to explain **if** the RNA that the test is looking for , **is not viral in origin.** Other confusing test results are listed in Appendix A.

Negative, Negative, Negative

A group of doctors in Marseille, France, working in a very experienced lab, that regularly does testing for respiratory 'viruses'??, reported testing 4,084 samples for the novel coronavirus, **using several systems approved for use in Europe, without a single positive** [25]. This included 337 people returning from **China** who were tested twice, and 32 people referred because of **suspected** coronavirus infection. It is statistically **improbable** that this lab was just lucky to **not** get any coronavirus cases, it is more likely that they **used more stringent criteria, illustrating that** the performance of not just the test kits, but **labs**, with this new test, is **completely unknown.** Yet, a positive test remains **unquestioned** in every case.

Test Experience

A paper from **Singapore** by doctors and public health officials provides a **revealing** look at the inner guts of coronavirus testing. **Hidden away in the supplementary material of reference** [24], where few people will see it, it **exposes some important issues with tests:** • The test is **not** binary (negative/positive) and has an **arbitrary** cutoff. • The quantity of RNA does **not** correlate with illness. • If negative means uninfected and positive means infected, then people went from infected to uninfected and back again, sometimes **several** times. • Results below the cutoff are **not** shown, and are treated as negative, but if PCR continued past the cutoff and was eventually positive, this would indicate presence of small quantities

of the RNA which is **supposedly unique to the** coronavirus (i.e. infection). Before you read beyond the following figure, ask yourself **why the first 6 graphs, shown deliberately out of numerical order, are separated.** What are the **visual differences** between those 6 and the remainder? Do this right away so my interpretation does not bias your opinion.

9 The Test is **Not** Binary

Tests for infections are usually reported as positive or negative (sometimes 'reactive' and 'unreactive'). One of the reasons for this is that, in many cases, multiple tests are required, and it is **common** to conclude that someone is infected **even with some negative tests** and that someone is **un**infected even with some positive tests. The results of a **complex multi-test** algorithm **are also usually reported as** positive or negative, **but interpreted by doctors and** patients as infected or uninfected. The **former** could mean isolation, special medications, special precautions for health care workers and more. But, in reality **even individual tests are not binary,** not positive or negative, **but a range of** numbers that are **arbitrarily** divided into positive on one side and negative on the other. Possibly there is a **grey** area that allows **other** factors, including the **bias** of the doctor or laboratory, to enter into the **interpretation**, or that will require further testing.

Understanding RT-PCR

Before we continue it is important to understand what RT-PCR, the test technology is. It is based on PCR (Polymerase Chain Reaction), a DNA manufacturing technique invented by Kary Mullis, who received a Chemistry Nobel for it in 1993. It is one of the **most important**????? technologies invented since the rise of the biotech industry in the 1980s. Starting with one DNA strand, the strand is cleaved (split in two) and then complementary strands are **allowed** to grow, the **same** process that occurs in a cell during mitosis (cell division). So far, **not so impressive, but through the magic of doubling, if this process is repeated 10 times you will have about** 1,000 identical strands of DNA. **Twenty** times, 10 a million (220). **Thirty** times, a billion (230). **Forty** times, a trillion (240). Each round of doubling is referred to as a **cycle**. **To use (or abuse)** PCR as a test, you **assume** that you are starting with an unknown number of strands and end up with an **exponential** multiple after n cycles. From the quantity of materials **at termination** the **starting** quantity can be estimated. A **major problem** with this is that because PCR is an exponential (doubling) process, **errors also grow exponentially.** In reality, the starting quantity is often **not** estimated, but the optical density, **or another** characteristic, of the growing pile of DNA, can be determined. Another **problem** with many **viruses??**, like Coronavirus, is that they are **believed??** to be composed

of RNA, but this can be **solved** by converting all RNA into DNA with the Reverse Transcriptase **enzyme** at the **start** of the process. **The technology, after these two adaptations, is known as** RT-PCR (Reverse Transcriptase PCR). Now you have the information necessary to understand the numbers from 20-40 on the vertical axis of the graphs above. These are the number of cycles. It **implies** that **it always took at least** 20 PCR cycles **before** any RNA could be detected, and they **stopped** after a maximum of 37 cycles. The blue line is at cycle 38, and the black dots do **not** mean RNA was detected after 38 cycles (as clarified in the paper), but that it **wasn't** detected by 37 cycles, and so the process terminated. This "Serial Cycle **Threshold** (Ct)" was the **arbitrary** definition of a **negative** result by the authors of reference [24]. We can see that it was **arbitrary**, because in **another** paper, reference [13], the authors had **two end** points: 37 and 40. Anything **less than** 37 was considered **positive** and anything 40 or greater was defined as negative. The in-between values of 38 and 39 resulted in **re**-testing. Note that **this** paper would treat 37 as **indeterminate** but the **Singapore** paper would treat it as positive. RNA Quantity does **not** Correlate with Illness **Theoretically** the PCR cycle number at which DNA is detectable tells us the relative quantity of RNA. Whatever initial amount was necessary to be detectable on the 20th cycle, 21 cycles would be **doubly sensitive**, and could detect about half as much, and 30 cycles about 1000th as

much as 21. One could therefore expect **sicker** people to have more virus??, and thus to have a **lower** cycle number on testing. This is the reason the authors separated out the first six graphs from the remaining twelve. The **first** six were the people who were **sick** enough to require oxygen. But one can clearly see from the graph that the **six sicker** people did **not** have distinctly **higher** quantities of RNA.

Positive to Negative and Back Again

The majority of the 18 patients had a positive test, followed by a negative test, followed by a positive test. Some had this several times. 11.. **If** a negative test means uninfected, then this is **impossible**. You cannot rid yourself of the virus, and then be re-infected the next day, and then infected the day after and uninfected again. The simplest answer to this conundrum is that negative tests do not mean uninfected. But the corollary is **that positive tests do not mean infected. Which would make the test worthless.**

Results Below the Cutoff

The authors of reference [24] apparently **programmed** the PCR machine to stop after 37 cycles if no DNA had been detected. This means that we **don't** have information on when or if the process would have terminated **if it had been allowed to continue** for many more cycles. More importantly, **what would it mean if** DNA was detected on cycle 38 or 40 or 80? **If** the RNA (complementary to

the DNA used in PCR) **is unique to the virus there is no other possible interpretation** than that the person is infected. But it is **possible** that everyone would eventually detect enough material, **which could only be interpreted as the corresponding RNA being endogenous (i.e. formed within the cells of the human body).**

Given that several people bounced back from negative to positive again, one could argue that the cutoff should be **more (or maybe less)** than 37 cycles. But likely if this was done many more people might test positive, and even with a cutoff of, say, 40, going to negative and back again might still occur.

7. Transmission

There is lots of evidence that the virus is not as transmissible as is being implied. (January 2) "27 (**66**%) [of 41 early] patients had **direct** exposure to Huanan seafood market [i.e. about **1/3 did not**]". [2]. (January 1-20) "Of the 99 patients with 2019-nCoV pneumonia, 49 (49%) had a history of exposure to the Huanan seafood market." [10] [i.e. 51% did not] (January 1-January 22) A **larger** survey, including all the first 425 cases, showed that of those diagnosed January 1st or later, 72% had "**No** exposure to either market or person with respiratory symptoms". [13] "The symptom onset date of the **first** patient identified was Dec 1, 2019. **None of his family** members developed fever or any respiratory symptoms.

No epidemiological link was found between the first patient and later cases." [2] (of the family cluster) **"None of the family** members had contacts with Wuhan markets or animals…They had **no** history of contact with animals, visits to markets including the Huanan seafood wholesale market in Wuhan, or eating game meat in restaurants." [3]

12 Transmission 1 – The Shenzhen Family Cluster
Reference [3] attempts to show the **ease** with which the **virus** could be transmitted in a family that travelled from Shenzhen, near Hong Kong, to Wuhan in December, and then back again about a week later. Two grandparents (patients 1 and 2), the daughter and son-in-law (patients 3 and 4), a 10-year old grandson and a 7-year old granddaughter (patients 5 and 6) flew to Wuhan on December 29th. On the first day, the grandmother (1) and her daughter (3) visited a baby boy with pneumonia, known as Relative 1, in a hospital in Wuhan (the hospital is **not named, but the implication is that** this child had this new disease). Outside of this they mingled with **four other** local relatives, of which two had also spent extensive time in the hospital. Notably the **infant's** symptoms resolved one or two days after the visit, and he returned home. On day **four** of the visit (January 1st), the son-in-law, who had **not** gone to the hospital **got** sick. **On this basis,** they **declared** that the coronavirus had a **very short** incubation time, and that **people were almost immediately infectious.** There's **no evidence** for this,

except nothing else can support their **hypothesis** that the hospitalized **baby had this new** coronavirus, infected Patients 1 (grandmother) and 3 (daughter), one of which then infected the son-in-law (Patient 4). **All in four days.** Then, like dominoes, the other visitors got sick, the daughter one day after her husband (Jan 2), the grandmother the next day (Jan 3), and then the grandfather and Relatives 2, 3, 4 and 5 (Jan 4). **The family appeared to have a history of being frequently ill.** In this case symptoms were mostly fever, cough and weakness. On January 4th the **whole** family returned to Shenzhen. Note that the grandchildren, patients 5 and 6, had **no** symptoms during their time in Wuhan, or after returning home. On January 9th, the grandparents and their daughter went to a **clinic** in Shenzhen, and the **next** day the grandparents visited the **big** hospital (University of Hong KongShenzhen Hospital) for tests. The daughter followed one day later (January 10th). **The grandparents had significant pre-existing health conditions, such as having been treated for brain cancer (grandmother) and hypertension (both).** In **Wuhan** they both suffered from fever, dry cough, weakness, and later were found to have **various lab abnormalities.** They were genuinely sick. Concern that they were infected with the **new** coronavirus is probably the reason why the **rest** of the family were brought in , over the next few days for testing. The daughter and son-in-law were still sick (diarrhea, congestion, sore throat, chest pain) but by then

had a normal body temperature (actually **lower** than 37C). They did have some **lung opacities** on a CT scan, so were diagnosed with pneumonia **despite** the normal temperature. The grandson had been a **bad** boy (patient 5) and had **refused** to wear a mask in Wuhan, so the parents insisted he get a CT scan. **Despite** the complete lack of symptoms, he also had lung **opacities**, and so was also diagnosed with pneumonia, albeit **completely asymptomatic.**

13 The granddaughter was a **good** girl (patient 6), and had worn a mask, and so nobody was surprised that she was **not** only asymptomatic, but also did **not** have lung abnormalities. All six patients (apparently including patient 6 who was **healthy** in all ways) were tested using the **new** RNA test. Not surprisingly, the grandparents tested positive on **nose** swabs and **serum** samples. The son-in-law tested positive on nose **and** throat samples. But the daughter, Patient 3, **despite** doing 18 tests, more than anyone else, stubbornly tested negative on each one. But, showing shocking **bias**, the authors concluded, "she was **still regarded as an infected case because she was strongly epidemiologically linked to** the Wuhan hospital exposure and radiologically showing **multifocal** ground-glass lung opacities." Another indication of **bias** was the **omission** of test results for Patient 6, who **also** tested similarly tested negative **every** time (but based on only four samples, according to personal correspondence from

the authors). In this case the **bias** was clearly **to classify her as uninfected.** The bbad grandson (patient 5) also tested **positive** on nose, throat and sputum samples, **despite** having no symptoms of illness. Additionally, there was a relative who did **not** travel to Wuhan (Patient 7), who got sick with back pain and weakness four days after everyone returned to Shenzhen and, when she was tested, she also tested **positive** for RNA (nose, throat and sputum). **Several** of the relatives who lived in Wuhan also got sick afterwards, **but no coronavirus test information was provided** in this paper. **No** consideration was given to **other causes for illness,** such as exposure to food contaminated by chemicals, food that was prepared in anticipation of their visit, that was left out too long, or in **unsanitary** conditions.
The purpose of reference [3] appears to have been **to prove that** the putative coronavirus is **infectious.**

Note that the relatives visited each other a lot over a few days, that was indeed the purpose of the trip.

Transmission 2 – The German Connection Reference [9] attempts to connect the illness of some **Germans**, one of whom met with a **Chinese** woman, who afterwards was diagnosed positive on the RNA test. The sequence of events **started** between January 20th and 22nd when a woman from **Shanghai** and a local German were in meetings together. Both were healthy at the time. The

woman flew back to China on January 22nd and started to feel sick on the flight home. The German also got sick (sore throat, chills, muscle pain, fever, cough), late on the 24th, and did not return to work until the 27th. By coincidence, this was the same day that the Shanghai woman informed the **German** company that she had been sick and had tested **positive** for coronavirus RNA. By this time the German man had **recovered without** any special medicines or interventions, but he tested **positive**, and so did **three other** colleagues who had contact with him, or the **Shanghai** woman, **or both.** It is logical that everyone who had any contact with them was tested, and likely **no** employees who did **not** have contact were tested. The paper does **not** say **how many tested negative,** and whether any of those testing negative had **similar** symptoms. The article claims that **all the four** Germans had symptoms starting on the 24th, 26th, or 27th, but what those symptoms were , is **not** detailed for the three , **not** in the meeting with the Chinese woman. The article does note that, "so far, **none** of the four confirmed patients show signs of **severe** clinical illness".

If the purpose of the paper was **to support** the idea that this illness is transmissible, it is important to accept the four positive tests on Germans as **true** positives, despite the fact that **none** of them had "severe clinical illness". This, however, calls into question the severity of the illness, and why heroic and **dangerous** medical measures

are needed. Because the **Germans** did **not** find out about their positive RNA test **until** after their period of symptoms, they probably **only** had to suffer quarantine, and **not** antiviral drugs, steroids or invasive respiratory assistance, which might have happened **if they had shown up at an emergency** department with symptoms **and** had been diagnosed with the 2019 coronavirus at the same time. An **alternative** explanation is that the coronavirus is **deadly**, but that these four Germans represent four **false** positive tests. If this is the case, the **usefulness** of the test must be questioned. Note that the fact that **all the people with positive tests and symptoms had contact**, is not surprising **if testing was limited to people who had contact.**

Transmission 3 – **Illinois** Couple

A paper in Lancet made a big deal about the presumed **first** case of person-to-person contact in the USA, from a woman who had visited **Wuhan** in December 2019, to her husband, who had stayed in the United States. She got sick after returning, and later both she and , her husband who had **not** travelled to Wuhan, tested positive for the coronavirus [31]. Whether he had symptoms or **not** was impossible to tell because **he had chronic obstructive pulmonary disease,** so had a cough **and** difficulty breathing **all** the time. What is more interesting is that authorities identified 372 **contacts** of this couple, and "were able to assess exposure risk and actively monitor

symptoms for 347". **Not one** of these people had an emergency room visit with **respiratory** symptoms within 14 days of contact with the couple. 43 **did** have some symptoms that could have been '**COVID**-19', and became "Persons Under Investigation" (PUIs). 26 had had exposures to the couple **classified** as "medium risk or greater". But despite the **presence** of symptoms, contact with the couple, and close monitoring, **not one** tested positive for the new coronavirus.

Transmission 4 – Diamond Princess [33] The Diamond Princess cruise **ship** was a perfect laboratory for watching a highly infectious pathogen in action. The **first** person who tested positive had **symptoms** , **before** boarding the ship on January 20th. It was **not** until February 1st that they tested positive, and February 3rd when passengers were confined to their quarters, in some cases **with** someone who tested positive. Passengers had interactions with the crew, e.g. to obtain meals. Despite this, the rate of transmission was only 16.7%, meaning that **83.3% remained negative.** Since almost half of those who tested positive had **no** symptoms , it was **not** possible to avoid contact with positive persons based on observing symptoms, and it meant that 92% emerged from quarantine without having experienced symptoms due to the coronavirus.

Transmission 5 – Magical Numerous newspaper articles have noted cases **outside** China (where individual cases were still newsworthy) that had **no** known contact with another case, or travel to an endemic region (notably Wuhan). These are document in Appendix B.

Proving Transmission

It is **impossible**, in most cases, to prove that someone did have contact with another coronavirus case, even if they did travel to Wuhan and visit the Huanan market. In the best case it will be possible to show that someone was in the vicinity of someone who tested positive earlier, but that does **not** constitute proof that they were **exposed** to the virus, let alone that it was that person who infected them. In most cases, even if someone was in Wuhan, there will be **no** evidence that a person was in contact with another victim. Fundamentally, this belief that , it is contact that causes positive tests , is necessary to preserve the infectious paradigm. Therefore, the slightest evidence of an association between an old case and a new case (such as having been in the same city at the same time) is taken as **proof** of transmission, **when it is obviously not.**

Preserve the test

Overall, it seems that **test results must be interpreted** to preserve the coronavirus **theory**. **No alternative interpretation is allowed.** And when there is an **inconsistency**, it must be ignored or explained away,

often invoking **imaginary** data. These situations are listed in Appendix C. 9.

Treatment

Treatment for the putative novel coronavirus is following the **same** pattern as for SARS. Apart from standard treatment for respiratory conditions, there is a tendency towards providing **oxygen** to patients **more aggressively** (e.g. intubation), the use of high dose corticosteroids (e.g. methylprednisolone) and a variety of antiviral medications.

SARS Experience

This did not work out well for SARS. As a report, commissioned by a WHO expert panel after SARS was over, said, **"Despite an extensive literature reporting on SARS treatments, it was not possible to determine whether treatments benefited patients during the SARS outbreak. Some may have been harmful** …Of patients treated with **ribavirin**, 49/138 to 67/110 (36%–61%) developed haemolytic **anaemia** [breakdown of red blood cells], a recognised complication with this drug, although it is **not** possible to rule out the possibility that SARS-CoV infection **caused** the haemolytic anaemia, as there is **no control** group. One study noted that over 29% of SARS patients had some degree of **liver** dysfunction indicated by ALT levels higher than normal, and the number of patients with this

complication **increased** to over 75% after ribavirin treatment…In the **Chinese** literature, we found 14 reports in which steroids were used. Twelve studies were **inconclusive** and two showed possible harm. One study reported **diabetes** onset associated with **methyl**prednisolone treatment. Another study (an **uncontrolled**, **retrospective** study of 40 SARS patients) reported **avascular** necrosis and osteoporosis among corticosteroid-treated SARS patients [which resulted in many joint replacements, particularly in Hong Kong]" [7].

Antiviral Drugs for Novel Coronavirus

For SARS the antiviral drug **ribavirin was dominant,** but for this **new** coronavirus, a **wider** variety have been proposed, **starting** with the Chinese at the beginning of the panic. The **choice** of drugs is a shot in the dark ,as, **"No antiviral treatment for coronavirus infection has been proven to be effective"** [2]: • Flu drug oseltamivir (**Tamiflu**) [2]. Use was described as "**empirical**", based on **intuition**, **not** science. Usage in China was also reported in [10]. • AIDS drug **combination** Kaletra, composed of protease inhibitors Lopinavir **and** Ritonavir, has been fairly **widely** used. A Chinese hospital noted that the choice was **because** the drug was "already available in the designated hospital" [2]. Usage in China was also reported in [10] and in **Singapore** in [24]. • Cytomegalovirus drug Ganciclovir (Cytovene) was also reported in China by [10]. • Early in February the Chinese

government announced a **trial** of Gilead's **Ebola** antiviral **Remdesivir**, on the **basis** that it, **"may have** helped alleviate the **symptoms** of a 35-year-old male" diagnosed with a coronavirus infection in the US [15]. The drug was going to be trialed on 270 people, although it is **not** clear whether there will be a **placebo** or comparison group.

A **Chinese** chemistry professor, Jiang Xuefeng, warned, **"No random, controlled, or blank samples were used in** [its previous use in one American man]…The effectiveness of **remdesivir** cannot be determined by this single case…It can take **years** to fully understand the pharmacological and toxicological side effects of new drugs"[15].

Reference [28] admits that, **"randomized and controlled trials are still needed** to determine the safety and efficacy of **remdesivir**." 17 • A **Japanese** hospital used the anti-**influenza** medication Avigan (Favipiravir) on one patient, and it was given to 70 patients in Shenzhen, China [30]. • A review of treatments in China, published mid-February, also **revived** the use of **Ribavirin** despite admitting **lack** of effectiveness and "**significant** toxicity". But perhaps, they **hypothesized**, it would be useful combined with other drugs [28] • **Nelfinavir** is another AIDS protease inhibitor, mentioned in [28]. • **Arbidol** is a Russian anti-influenza drug, mentioned in [28]. • **Nitric oxide** inhalation is also mentioned in [28]. These drugs

are sometimes described as "**experimental**", but that is a **misnomer**, and **disguises** the fact that they are **not** used in the context of **science**. It is clearly **not** science when there is **no** placebo, **no** blinding, and **no** randomization. It is likely that **sicker** patients will be prescribed **untested** drugs, if they have a health **decline** it will be **blamed on the virus,** and nobody could know what would have happened **if** they had received standard medical treatment for their symptoms. **If** the patient survives it will likely be considered a success, and is worth millions, or more, in public relations to an antiviral drug that has not yet found a market.

Drug Usage

93% of 41 **confirmed?** Chinese coronavirus cases in [2] received Oseltamivir, and **future** use of Kaletra was planned. 75 of 99 patients, also in China, received **unspecified** antivirals [10]. 52% of 2,003 coronavirus positive people who **died** in **Italy** had been prescribed (**unspecified**) antivirals [32].

Patient Status

Part of the concern with the use of antiviral drugs is that for this epidemic panic the patients are **older and weaker than** the average person. For example, "The average age of the patients was 55.5 years, including 67 men and 32 women" [10]. **Only** about **12**% of the Chinese population are 55 or over [11]. In a later study [13], the median age

was 59, and **only** about **10**% of Chinese are this age or older. In the last of three time periods of this study, January 12th through 22nd, the median age had crept up to 61. The patients were also **weaker**. For example, "50 (51%) patients had **chronic** diseases, including **cardiovascular** and **cerebrovascular** diseases, **endocrine** system disease, **digestive** system disease, **respiratory** system disease, **malignant** tumour, and **nervous** system disease" [10]. In Italy, the average age of 2,003 analyzed deaths was **80**, and over **99**% of the 355 cases for which this information was available had **serious** pre-existing health conditions (76% had hypertension, and smaller quantities had diabetes, other heart conditions, recent cancer, kidney failure, COPD, stroke, dementia and chronic liver disease) that could have explained the deaths [32]. **49**% had over three conditions, 26% had two and 25% had one. Only three of the deceased (d (<1%) had **no** chronic underlying condition.

Combine old age, pre-existing health conditions, pneumonia, invasive ventilation and powerful drugs, and you have a recipe for another iatrogenic **disaster**.

Treatment experience

We do **not** have much precise **documentation** of experiences with the antiviral drugs, this tends to come out **after** an "epidemic" is over, when doctors have time

to go through the copious records that will be taken, and see if they can determine whether the treatments had any impact on the markers of the disease or on the health of the patient. Since it is almost certain that there was **no control**, it will be **impossible** to distinguish between a patient who recovered **on their own** despite the treatment, and one who was saved by the treatment. However, useful information on adverse events and disease markers can be obtained. The first report of treatment experience that I am aware of came from Singapore [24]. They reported on 18 patients, of which **only** five received antiviral medications, chosen from six **sickest** patients, who required supplemental oxygen?. The doctors used the AIDS drugs **Lopinavir** and **Ritonavir**, often marketed as the combination pill Kaletra. For two of the patients they reported a reduction of oxygen requirements? within 3 days, and for two they started to get negative coronavirus tests (not the same two). So far, so good, although it is impossible to claim this is due to the drugs, and it was only a minority of the patients. The **bad** news is that two patients, "**deteriorated** and experienced progressive respiratory failure while receiving lopinavir / ritonavir, with 1 requiring invasive mechanical ventilation". And these two patients **continued** to produce positive coronavirus tests. Furthermore 3 out of 5 patients "developed abnormal **liver** function test results" and 4 out of 5, "developed nausea, vomiting, and/or diarrhea". In total, **only one** of the five was able to complete the

planned 14-day course of antiviral drugs. It is of course **not** possible to prove that the drugs produced the side effects or worsening of health that is seen, as a **control** is **im**possible during an epidemic panic. However, when the condition of the patients includes **known** side effects of the drugs prescribed, it is reasonable to infer that they are caused by the drugs.

10.Conclusions

The coronavirus panic is just that, an **irrational panic, based on an unproven** RNA test, that has **never been connected to a virus.** And which won't be connected to a virus unless the virus is **purified**. Furthermore, **even if** the test can detect a novel virus the presence of a virus is **not** proof that it is the cause of the severe symptoms that **some** people who test positive experience (but **not** all who test positive). Finally, even if the test can detect a virus, and it is dangerous, we do **not** know what the rate of false positives is. And even a 1% false positive rate could produce 100,000 false positive results just in a city the size of Wuhan and could mean that a significant fraction of the positive test results being found are false positives. 19 The use of **powerful** drugs because doctors are convinced that they have a particularly potent virus on their hands, especially in older people, with pre-existing health conditions, is likely to lead to many deaths. **As with SARS. There is very little science happening.** There is a rush to explain everything that is

happening in a way **that does not question the viral paradigm, does not question the meaninglessness of test results,** and that promotes the use of **untested** antiviral drugs. And, given enough time , there will be a vaccine developed and, for some of the traumatized countries, it may become mandatory, even if developed after the "epidemic" has disappeared, so that proving that it reduces the risk of developing a positive test will be impossible.

'Magical' Positive Cases

The following cases are gathered from news reports of people who tested positive with no known connection with other coronavirus cases, plus no travel to the originally affected regions. To avoid cluttering the reference list, the references are not provided, but are available upon request. • (Feb 2) An 80 year old Hong Kong man tested positive after hospital admission due to a fever, but his only recent trip to mainland China was a brief visit to Shenzhen, just outside Hong Kong (over 1000km from Wuhan by car). He had no contact with other cases, markets with live animals or wild animals. • (Feb 13) A Japanese woman in her 80s tested positive after death. Her son in-law, a taxi driver, also tested positive. He had not travelled to the affected parts of China and denied having carried any foreign customers in the two weeks before testing positive. • (Feb 16) An 82-year old man in Seoul, Korea, had no record of overseas

travel or contact with other positive testing people. • (Feb 17) Three men in Aichi, Chiba and Hokkaido prefectures in Japan have no infection routes identified. • (Feb 18) A 61-year-old woman described as a "**superspreader**" was the **first** person diagnosed in her **highly** populated region of South Korea, with no known contacts or travel to explain her case. She was **blamed** for spreading the infection to 37 other people, but this may just be an artefact of the size of the church. She had 1,160 "contacts" (presumably mainly members of her congregation), and so the fraction of cases among her contacts is **3.3**%, lower than the rate of positive tests seen overall in South Korea. • (Feb 22) Two cases in Chiba prefecture, Japan, had **no** relationship with each other, or any contact with other cases or a relevant travel history. • (Feb 22)

Director-General of WHO says that "cases with no clear epidemiological link, such as travel history to China or contact with a confirmed case" are a concern.

• (Feb 24) In Lombardy, Italy, none of the early patients had been to China or had contact with another case. • (Feb 27) After a hospital in Vienna, Austria, decided to test everyone with compatible **symptoms**, a 72-year old man tested positive. He had no known route of infection, had already been in the hospital 10 days, and none of his contacts were ill or infected. • (Feb 27) An 88-year old

man in San Marino (Duchy within Italy) tested positive, but an investigation showed he had not travelled abroad, nor to the '**red'** areas of Italy where other cases have been found. • (Feb 28) An Oregon resident became the first positive case with no known history of travel to affected countries or contact with infected individuals. • (Mar 2) El Pais reported that at least five positive cases in Torrejón de Ardoz, near Madrid, had not travelled to any country considered a risk, not had contact with any other patient. 24 • (Mar 6) British Columbia, a, Canada reports a positive case with no recent travel history and no known contact with another patient. • (Mar 19) CTV reports on a man with **leukemia**, who went to a hospital with night sweats and a cough, was given antibiotics and sent home, worsened, was intubated, and then sadly died. A test result received after his death was positive. He had not recently travelled or had contact with another coronavirus patient.

Appendix C Preserve the Test Overall, that test results must be **interpreted** to preserve the coronavirus theory. No alternative **interpretation** is allowed. And when there is an inconsistency, it must be ignored or explained away, often invoking **imaginary** data. • As mentioned above, in Reference [3] the **daughter, important in the chain of transmission of a family, was interpreted as a false negative.** Alternatively it **could have been** concluded that this woman did **not** have the coronavirus, but that

would have **badly damaged** the family transmission story, and **left open other reasons for the cluster of illnesses** (and CT scan abnormalities). • Also in Reference [3] the grandson tested positive without any symptoms at all, except lung abnormalities on a **CT** scan. This allowed them to **declare** him as ill (asymptomatic pneumonia). But he **could have been** an asymptomatic case or a false positive. • A woman who returned from China to her Canadian university with illness, first tested negative, and then positive. This was **interpreted** as indicating that she had very little virus in her body at the time of the first test, and that the test was **not** sensitive enough. However, PCR testing is **extraordinarily** "sensitive", and **if** she had **so little** virus, how was it that she had symptoms? An alternative explanation is that she became positive on the test in Canada, perhaps because this virus is actually quite common, or because the test is **not** for a virus, but is just measuring RNA? **created** by the human body in response to disease conditions.[8] • The four Germans [9] could be seen as showing that the RNA test **produces** false positives or that the illness produced by the virus is often not severe. But it will be **interpreted** as **neither** , by dogmatic promoters of the coronavirus **theory**, it simply will **not** be mentioned , now that the main message, that the virus is infectious, is bolstered by the evidence. • Out of **206** Japanese evacuated from Wuhan, only **three** tested positive, and **two** were found to have "no symptoms". **Instead** of considering them false positives, they are

considered infected and possibly infectious.[12] • Of 6 positive cases in Singapore reported in [14], the first had a sore throat and cough, but no pneumonia, the second and third had undescribed symptoms, and the last three had no symptoms. • A woman returned from Italy to Cuba, where her husband was, and developed minor respiratory symptoms after her return [27]. Her symptoms resolved, but a few days later the husband developed symptoms, and both went to a hospital, where they were put in isolation. When they were tested, **the husband, who had not been outside the country, was positive, but the wife was negative.** The medical institute **hypothesized** that she had become negative in the 15 days after her first symptoms, but there **was no evidence that she had ever been positive.**
http://theinfectiousmyth.com/book/CoronavirusPanic.pdf

Media's hysteria and the politicians' rhetoric is going pandemic.

German immunologist and toxicologist, Professor Stefan Hockertz, explains in a radio interview that Covid19 is no more dangerous than influenza (the flu). More dangerous than the virus is the fear and panic created by the media and the „authoritarian reaction“ of many governments.

Professor Hockertz also notes that most so-called „corona deaths“ have in fact died of other causes while also testing positive for coronaviruses.
Hockertz believes that up to ten times more people than reported already had Covid19 but noticed nothing or very little.

SYMPTOMS MEAN NOTHING ANYMORE. THE SCIENCE ESTABLISHMENT WILL DECIDE , WHO HAS WHAT , WHETHER HE SHOULD BE CULLED OR QUARANTINED .

The Argentinean virologist and biochemist Pablo Goldschmidt speaks of a „global terror“ created by the media and politics. Every year, he says, three million newborns worldwide and 50,000 adults in the US alone die of pneumonia.

Professor Julian Nida-Ruemelin, former German Minister of State for Culture and Professor of Ethics, points out that Covid19 poses no risk to the healthy general population and that extreme measures such as curfews are therefore **not** justified.

Using data from the cruise ship Diamond Princess, .. the age-corrected lethality of Covid19 being+ is between 0.025% and 0.625%, i.e. in the range of a strong cold or the flu.

Moreover, a Japanese study showed that **of all** the test-positive passengers, and despite the high average age, 48% remained completely symptom-free; even among the 80-89 year olds , 48% remained symptom-free, while among the 70 to 79 year olds it was an astounding 60% that developed **no** symptoms at all.

The Italian example has shown that 99% of test-positive deaths had one or more pre-existing conditions, and even among these, only 12% of the death certificates mentioned Covid19 as a causal factor.

"The electron microscope is capable of revealing details as much as 1000 times smaller than visible , in light microscopes because the wavelengths of **electrons?** are much shorter than those of light. **((what is electron ? what is their wavelength ??))** Transmission electron microscopes make it possible to explore **cell??** structures and large protein molecules??. Because beams of electrons pass through thin samples, cells and tissues.

Transmission Microscope … MUST BE CUT INTO THIN SLICES BEFORE THEY CAN BE EXAMINED UNDER THE MICROSCOPE.

With **scanning** electron microscopes, a pencil like beam of electrons is scanned **over** the **surface** of a specimen. For images, specimens do **not** have to be **cut** into thin slices to be visualized. The **scanning** electron microscope produces **scanning** three-dimensional images of cells. Because electrons are **easily scattered by** molecules in the air, samples examined in both types of electron microscopes must be **placed in a vacuum** in order to be studied. As a result, researchers **chemically preserve** their samples first and than **remove** all the water before placing them in the microscope.

THIS MEANS THAT ELECTRON MICROSOPY CAN BE USED TO VISUALIZE ONLY NON-LIVING PRESERVED CELLS AND TISSUES."

One doesn't have to be a scientist or doctor to understand the way an electron microscope works. Any intelligent 10 year old can understand the words,

THIS MEANS THAT ELECTRON MICROSCOPE CAN BE USED TO VISUALIZE ONLY NON-LIVING PRESERVED CELLS AND TISSUES."

No one has EVER seen a live virus or **antibody**, **because they don't exist.** It is all a **scam promoted by Thomas Rivers,M.D. in 1927.**

The word **virus** is from the Latin and means poison. Here is the evidence of what the virus really is and where it comes from. The virus that was **promoted as the cause of** polio and other diseases , was just the poison or infection taken from a person or animal **with** a disease. The poison or infection **was not the cause of itself** --it was the **result** of disease and therefore **cannot** be a prevention or cure of a disease.

Straight from the liars who taught the virus lie to everybody. And there is your "live virus." The pus of a smallpox sore. Poison.
https://alternativehealthadvice.blogspot.com/2020/03/no-one-has-seen-living-virus.html

In **2009**, was **Swine Flu** pandemic. In the summer of 2009, the Centers for Disease Control, **ignoring** their federal mandate, **secretly** stopped counting Swine Flu cases in America. Yet they **continued** to stir up fear about the “pandemic,” **without** having any real measure of its impact.

Leading nations of the world want:
--- more control over their people
--- an easier way to eliminate their opposition
--- a cashless society
--- an excuse to take away property of dissenters
--- an elimination of the means of self-defence

--- a method of reducing world population, i.e.**Agenda21**

Having a pandemic is a perfect cover-up for all of this. Also, it allows pharmaceutical companies to make 10's of billions more – perhaps a trillions of dollars.

--- Bird Flu, and Coronavirus. Notice a **pattern**? There are more **patterns**. Did millions die from the Bird Flu?

"I have lectured all over the world... I have always had a special interest in **newspapers**. **All of them have one thing in common, there is always some reference made to some epidemic in some part of the world.** For instance, two years ago, one paper referred to a **polio** epidemic in Holland. For the past three years, our newspapers have commented on the **diphtheria** epidemic in Russia. By these means, the **population is constantly threatened with epidemics,** they have been made to fear them, and the reports always conclude: "Go and get vaccinated".--Dr Buchwald MD

"Dr.Martin Hirte writes on page 20 of his book 'Vaccination--Pro and Contra': **"To create fear among parents** to strengthen their motivation to vaccinate is an **important part of the publicity used to promote** vaccinations.

A whole branch of research is examining the question:

'What level of fear needs to be created to appear as convincing as possible?'"---Dr Buchwald (The Decline of Tuberculosis despite "Protective" Vaccination by Dr. Gerhard Buchwald M.D. p104)

- Vaccines: The Biggest Medical Fraud in History - Trung Nguyen, Eleanor McBean, Sue Martson – 2018

"Yet our medically controlled Health Boards cook up fake epidemics, create panics for profit, such as the ones in Kansas City in 1921, Pittsburgh in 1924, Philadelphia, Baltimore, Washington in 1925. An **effort** was also made to create a panic in New York in 1925, but due to the open fight against it by the New York Evening Graphic, the Commissioner of Health called it off."--Herbert Shelton DC

"Since people cannot be vaccinated against their will. the biggest job of a health department has always been, and always will be, to persuade the **unprotected** people to get vaccinated. This we attempted to do in **three** ways: first by education; second, by **fright**; and third, by **pressure**. We dislike very much to mention fright **and** pressure, yet they accomplish more than education, because they work faster than education, which is normally a slow process.

During the months of March & April we tried **education**, and vaccinated only 62,000. During May we made use of fright **and** pressure, and vaccinated 223,000 people.

Our **educational** program consisted of warnings in the daily papers, small-pox posters on the streets, in stores and factories, special small-pox bulletins for all large places of employment, and special letters to all large employers from the health department and the association of commerce, calling their attention to a threatening small-pox epidemic. The radio was also made use of in this work.
As the conditions grew worse, we felt justified in using stronger measures. We had some good **pictures** taken of patients suffering from the confluent type of small-pox, and had posters, showing these pictures, distributed all over the city. The moving picture theatres cooperated at this time by issuing warnings on the screen.

The newspapers published daily the names and addresses of people dying from **small**-pox. A second letter was sent to all factories, stores, and other places of business, informing them of a **rapidly** approaching small-pox epidemic, and advising them to have their employees vaccinated immediately, and thereby prevent a serious financial loss to the city, which might occur if a real epidemic developed.

At this time the department was vaccinating thousands of people daily, but there were still too many who could neither be educated **nor** frightened into vaccination. Cases and deaths each amounted to a considerable number, and we now felt **justified** in using all of the power a health officer has, and if that was not enough, to get more.

We sent out a third letter to all employers requesting them to have all of their employees vaccinated and at the same time informing them that if a **small**-pox case developed in their place of employment in the future , we would consider their place of business a menace to the health of the community and very likely place the entire establishment under **quarantine** until it could be cleaned up and made safe for the public. Putting this responsibility on the employ**er** drove in thousands of anti-vaccinationists who could better afford to get vaccinated **than** lose their jobs. All employees co-operated very bravely with this last request, although in a few instances it was necessary to lay off old, reliable and valuable employees."-----Declaration by Dr John Koehler, Commissioner of Health of Milwaukee, Wisconsin, in an article in The Wisconsin Medical Journal, November, 1925. (The Facts **against Compulsory** Vaccination by H. B. Anderson, 1929.)

[2010 Sept] **Pertussis** epidemic? **Or Media induced malady?** by Hilary Butler

Being an information junkie, I have a huge collection of newspaper articles about kids with pertussis dating from the late 70's, and... most of them are vaccinated children. One more recent prominent one was a 10 month, fully vaccinated daughter of a doctor at Middlemore hospital. Which reminds me of this medical article in which in 1979, while defending his article the author replied with a situation which **exactly mimics** THIS country: **I did discover that the only seriously ill infants were too young to have been vaccinated in any case;** and that these children were **threatened** by an **outbreak** of which the onset **and** initial spread were **entirely among "immunized" children.**

Nothing has changed in the world of expedient misinformation.

By 1976, the number of cases nationwide had dropped to 1,010 a year, because of what experts say was the widespread use of the vaccine. But the numbers have **increased** tenfold since. In California alone, there have been more than 4,000 cases reported this year, including nine infants who've died – most of them after being misdiagnosed initially, when the truth is, that because of "observer **bias**" and the **assumption** that all vaccinated children can **never** get pertussis, pertussis was constantly diagnosed as something **else**, **until** the truth gradually dawned on everyone, and that truth then permeated the

medical literature. Ironically, the **guru** of pertussis, Dr James Cherry, was the first to twig that , the problem was blinkered doctors, **not** absence of pertussis!

Why did that truth gradually permeate the medical literature? **The whole truth and nothing but, is never told in the medical literature about anything**.....In order **to convince** the public that pertussis boosters from cradle to grave is a good idea, you have to have time to concoct some plausible **sounding** excuses. As well as allow enough time to pass, to get away with absolute porkies.
But the mainstream media will **never** wake up to the fact that media induced maladies exist, because they **assume** that doctors tell the truth, and they **can't** be bothered studying the medical literature (or wimp out by saying they don't have the time).

Even if they did all the above, I wonder who would in mainstream media would have the balls to challenge people like Dr **Alison Roberts,** and demand truth and accountability?

ON PAST EXPERIENCE, THE ANSWER WOULD BE "NONE".

Definition of the word virus before it was changed

Webster's Shorter School Dictionary. 1927. virus, n. The poison **of** an infectious disease.

Thorndike Century Dictionary. 1935. 1. venom.2. A poison produced **in** a person suffering **from** a disease, as small-pox virus.

Webster's New Practical Dictionary. 1951. 1. venom. 2. The poison matter **of** a disease, as the virus of small-pox.

Webster's Encyclopedic Dictionary of the English language. 1957. virus. 1. venom. 2. A poison that is produced in the body **by** a disease.

"A poison that is produced **in** the body **by** a disease." The poison that is produced by the body in **every** disease is the infection or mucus. **The mucus or infection is the virus.** The infection or the mucus **is also the result of disease.** The infection or mucus **is what they make their vaccine out of.** So they make there vaccine **out of the result of disease not the cause of disease.** This is isopathy **which means** the product of disease will prevent disease. Isopathy was coined in 1823 by **Dr. Joseph Wilhelm Lux**. Dr. Lux named it after **Jenner** who died in 1823. I would say it was **a tribute from one quack to another.**

Let's think about this poison that the body produces in a diseased state. Using the true definition of the word virus, read that as small-pox pus. The pus is the poison produced **by** the body **in the disease** called small-pox.

Jonas Salk said vaccination is easy to understand. The vaccine is made from the virus that **caused** the disease. As you can see from the above true definitions Salk was **deceptively** referring to the infection **of** the disease. He was making his vaccine **out of the result** of disease, **not the cause.** He knew better.
Edward Jenner, the man who is credited with the filthy practice of vaccination in the 1800s used the pus from the smallpox lesion and said **if** placed in the belly of a cow and **then** placed the cow pus in the arm of a healthy person, that it **would prevent** smallpox.

Today all vaccines are **still** made **from the result of** a disease and claimed **falsely** to prevent disease. This is why the medical profession **hasn't cured** anybody in 2000 years. It is **like** saying a cancerous tumor is the cause of itself and then making a vaccine out of the cancer infection and claiming it will prevent cancer. Does that make sense ? of course not , **it is ridiculous.**

The **real** scientific term is **not** vaccination, nobody uses cow pus anymore, **it's Isopathy which means the**

product of disease will prevent disease. How stupid is that?

Thomas Rivers in 1927 **began** to **change** the meaning of virus , to today's B.S. definition.

Martinus Beijerinck and **Ivanovski** discoverers of virus. These are the men who took dying tobacco leaves, ground them up into a liquid, ran them through a **filter**, then injected the poison into the leaves of young geowing plants and **when** the plants changed color, **declared** that something in the poison **came alive and attacked the plants.** How frecking stupid was that?

It is dangerous to be right on a subject which the established authorities are wrong. -Voltaire

This page on the **so-called polio virus** was taken from a book written by **Alton Blakeslee** in 1956. Blakeslee was a press writer for the Associated Press. The book covered **every detail** on how Jonas Salk made his polio vaccine. Every detail **but one.** The **source** of the so-called virus was **not** named.
See the source below under Simon Flexner. **It was ground up spinal cord from a human child.** No I am not kidding.

The first and only question to ask yourself is , if they couldn't see the virus , how did they know it existed?

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Swine Flu & Fake Epidemics: Push for Global Management By Jon Rappoport

The Council on Foreign Relations (CFR), headquartered in New York, is one of the key power centres pushing Globalism for All. **Medical programs are a clever and deceptive strategy for advancing this goal – the coagulation of Earth under one system of political management.**

On October 16, **2009**, the CFR held a symposium titled: Pandemic **Influenza**: Science, Economics, and Foreign Policy.
Much of the information in this symposium report is window dressing. However, **it's worth** noting a few comments made by presenters:

"Laurie Garrett, senior fellow for global health at the Council on Foreign Relations, said at the October 16, 2009 New York symposium that amid **the array of unknowns** surrounding the H1N1 **virus**, **one certainty is that 'this is a worldwide event and it is occurring in the dawn of our age of globalisation.'**

Garrett added, 'It's a darn good thing , we are dealing with **a relatively mild flu this time,** because clearly we are **ill-prepared** at this moment for a more virulent or more dangerous virus, either **if** this one takes on a more dangerous form… or **if** a second totally different virus does emerge.'

Helen Branswell of the Toronto-based Canadian Press agreed: 'We thought we were preparing for a more serious (bird flu H5N1) issue, **but we are in fact not prepared for a mild one."** (Pandemic Influenza: Science, Economics, and Foreign Policy, Symposium Rapporteur Report, October 16, 2009, www.cfr.org/content/publications/attachments/LG_Pandemic_Symposium_101609_rapporteur_report.pdf)

So **two points were established** early on: the Swine Flu is a **mild** disease, **not a pandemic** by any sensible definition; and leaders of "our age of globalisation" must be prepared for a **more drastic disease event** by taking worldwide measures now.

This **latter** issue is **highlighted** by another contributing CFR speaker:
"It was the overarching **consensus** of the symposium, first forwarded in the gathering by Financial Times correspondent Andrew Jack of London, that the current

pandemic **must serve as 'a teachable moment',** focusing expert attention on the inherent contradictions in global governance of health issues, inequities in world access to vaccines and medical supplies, weaknesses in planning and management of epidemics with worldwide risks for economics and politics, and the public's respect for science and public health."

Andrew Jack thus punches up the notion that solutions to so-called global health problems **can only be attained through international means.**

Medical Crises and Global Governance

The report continues: "[Robert] Rubin [former US Secretary of the Treasury and Co-Chair of the CFR] noted that the increased global interdependency of the current economy **has changed the game for pandemic** responses in the United States, leaving only one option: 'If the United States, and the world global economy, is going to be moderately well-prepared for this, there has to be an enormous amount of planning and agreed-upon processes and regimen decisions before the [pandemic] hits'."

These speakers are talking about a **vast** system, a medical bureaucracy that can oversee planning and execution of "epidemic control" on a **global** scale.

Laurie Garrett then makes a pitch for equitable **redistribution** of wealth among nations:
"Moderator Garrett said: 'We have globalised [epidemic] risk and threat today, but not globalised **benefits**. So the whole world shares the risk of pandemic influenza, but **only a small percentage share vaccines, medicines and treatments'."**

Who would make those wealth-redistributing decisions from the top? **Who** would allocate money and drugs and vaccines and doctors from Greenland to Tierra Del Fuego? There is **only one** answer: an internationally organised body that could **override** the wishes of sovereign countries.

Then **John Lange** sounds a sour note of failure in this regard:
"In face of profound scientific and economic insecurities, important foreign policy decisions must be made by the United States to address the globalisation of pandemic protection and **benefits**, as well as threat. Ambassador **John E. Lange, of the Bill and Melinda Gates** Foundation and **former** Special Representative on **Avian and Pandemic Influenza** for the State Department, said international coordination in response to the H5N1 pandemic [another mild flu season] of the 1990s **paved the way for** today's response to H1N1. Nevertheless, Lange said, little has been done to move towards a more

institutionalised **global** response, due as much to a lack of political will as to strained resources, in spite of high expectations."

Lange thus draws the problem. The US has lagged behind. The US is **not** eager enough for "a more institutionalised global response." The US doesn't want to cede power to some agency like the World Health Organisation (WHO).

Another speaker takes off the **mask** and drives home Lange's point harder:

"Canadian Press' Branswell doubted how feasible it will be for countries such as the United States and Canada to deliver on these expectations. At the heart of the debate is the issue of sovereignty, which may prevent states from carrying through with their agreements in the face of pandemic pressure, instead choosing to nationalise local supplies of vaccines, masks, protective gear and other medical supplies. Conversely, sovereignty has been invoked as the **basis for refusing** to share samples of dangerous flu viruses with WHO and international scientists, and , for **declining** outside **inspections** of local outbreaks."

Surrendering National Sovereignty

Well, there it is. It doesn't take a genius to read between the lines. The surrender of national sovereignty is **necessary** if the world is going to deal with encroaching waves of pandemics. **Nations will have to give up their independent status in these situations – and you can be sure that the international body formed to govern epidemic disease will be permanent.** No one is stupid enough to think that the enormous effort and time and money needed to establish such a bureaucracy would fade away after the latest and greatest pandemic. **Control** would transfer now and in the **future**.

Medical crises, in this way, **translate** into further steps along the way to **global governance.**

Before citing more statements from the CFR symposium, let me offer some numbers on these "waves of world illness" we have endured over the last 15 years or so. Keep in mind that **epidemics are the primary justification for internationalisation of a medical monarchy.**

Total cases and deaths:
SARS – 8,096 cases – 774 deaths.
WEST NILE – 27,836 cases – 1,088 deaths.
BIRD FLU – 262 deaths.
SWINE FLU – On April **26**, 2009, with **20** cases of Swine Flu in the US and **no** deaths, the US Dept. of Health and

Human Services **declared a nationwide public health emergency.**

The WHO **changed its definition of pandemic so that "enormous numbers of deaths and illness" was removed from the definition.** This happened in May 2009.

Thus far, WHO **estimates** about 8,200 deaths from Swine Flu, **worldwide**. That would **average?** out to about 15,000 deaths for the year. **But** the CDC claims **36**,000 people die every year from **ordinary** flu in the US alone.

So far, the global count**?** of Swine Flu cases is 587,653**?**.

Yet WHO states, "Every winter, **tens of millions** of people get the [ordinary] flu. Most are only ill and out of work for a week, yet the elderly are at a higher risk of death from the illness. We know the worldwide death toll exceeds a few hundred thousand people a **year**…"

Fear Mongering New Diseases

So **why is Swine** Flu a pandemic, and **why is ordinary** flu **not** a pandemic?

Fear mongering is about NEW diseases. That's why.

It gets worse.

In early November, an explosive report by Sharyl Attkisson hit the CBS News website: Of all the probable

or suspected swine flu cases in California **actually tested by state labs since** July 2009, based on 13,704 tests, **only 2% of** the patients had**?** Swine Flu. 12% had **some other** kind of flu. And a whopping **86**% **didn't** have flu at all.

In Florida, based on 8,853 tests for suspected/probable Swine Flu, only 17% had**?** Swine Flu. 83% were negative for other flu. So 83% **didn't** have ANY kind of flu.

In Alaska, based on 722 tests for suspected/ probable Swine Flu, only 11% had**?** Swine Flu. In Georgia, based on 3,117 tests, only 2% had**?** Swine Flu.
My point here is this: **All these recent "epidemics" have been outright fakes.** The numbers of cases and deaths are miniscule compared with **older** traditional illnesses – for which no pandemic emergencies have been declared.

Therefore, when the CFR is talking about **globalising** pandemic responses, **and nations surrendering their sovereignty, it's all based on an epidemic cover story that is patently false.**

Continuing now, with the CFR symposium report: We come to the **toxic** portion of the issue. In many nations, there have been vigorous debate over the use of so-called **adjuvants** in flu vaccines. One such substance, **squalene**, has been banned in several countries, because it can have **dangerous** effects. But the CFR would apparently like to

override this question and promote universal use of **squalene** in vaccines, despite the glaring fact that Swine Flu itself is so **mild** , the **risks of the vaccine far outweigh its need.**

"While recently the Obama administration brokered a deal among **eleven wealthy** nations to donate 10 percent of their vaccine supply of H1N1 to WHO , for use in developing countries, Canada has **not** signed on, in an uncharacteristic decision… On the other hand, the Obama administration has **refused** the use of adjuvants, which are used in Europe, Canada and Japan to stretch out the **antigen** supply for wider global use, **causing** Lange to question the role of the United States as a true 'global player'. Adjuvants help **trigger** the immune response, allowing **dilution** of precious flu antigens so that upwards of **ten** times as many people can be immunised with the same antigen supply. **If** the US were using adjuvant in its H1N1 vaccines, the country could be in a position to offer sufficient **surplus?** product to WHO to bring the agency's supply for poor countries up by **hundreds of millions of doses."**

Not "a true global player." That epithet carries considerable weight in CFR and allied circles. It means, "Let's watch this person. If he wants our support, he's going to have to change his tune. Let him understand that."

Combating “anti-vaccine hype”

Finally, the CFR report takes a swipe at people who are educating **themselves** on the historical toxicity of vaccines. And here, it does**:**
“The public perception of swine flu has further complicated the issue, causing both public doubt and panic at the same time. Branswell fears that ‘the WHO has lost control of the message’, allowing **mis**informed threats, such as the current **anti**-vaccine hype, to resonate around the world as the scientific community races to catch up with the facts.

“The last great flu pandemic of **1968** occurred in a deeply **divided** world, where **entire** regions of the planet were **no**-travel zones for billions of people. It was an era of telephones and posted mail, evening newscasts, and morning newspapers. Both **viruses** and information spread comparatively slowly.

“Though today the vaccine methods of production and distribution mirror those practiced **a half-century ago,** the age of globalisation has ushered in rapid human and animal travel, leading to **worldwide** spread of viruses. The internet has similarly opened the door to viral spread of disease truths, half-truths and outright lies. Thankfully, the **mild** H1N1 has offered the world community an

opportunity to see these 21st Century challenges **without** simultaneously experiencing worst-case outcomes. It is a teachable moment, but it remains to be seen whether – on both global and local scales – governments, companies and individuals are learning."

Twenty years ago, when I was writing my first book, AIDS INC., I realised that medical **propaganda** could be used as a pre-eminent tool in **controlling populations, because** doctors and public health bureaucrats exude an air of political neutrality.

These "esteemed" figures **appear** to have **no** agenda of a political or economic nature. They speak as minor saints. They always "care and share." When they say citizens must take certain actions to protect themselves and their loved ones, they speak with great authority.

Under that flag, much destruction can be wrought. For example, in certain areas of Africa, people have been dying from the **same** causes for hundreds of years: protein-calorie malnutrition; outright starvation; gross lack of sanitation; overcrowding; contaminated water supplies; abject poverty; no hope; and more recently, vaccines **and** medical drugs which, administered to people whose immune systems are **already devastatingly compromised, can be lethal.**

At the root of these causes is stolen land. Colonisation by governments and then mega-corporations, and brutal repression by **local** dictators – such controllers want to conceal their own naked actions, and they also want to keep **hidden** the actual immediate **causes** of death in Africa – the causes they- the controllers, invoke and maintain.

What **better way** to reframe this incriminating picture **than to claim that** a few **politically neutral germs** are the agents of death. Then, you can build a few **showplace hospitals,** bring in a bevy of doctors, set up a lab or two and demand that pharmaceutical companies donate medicines for the suffering. Meanwhile, **no one** cleans up the water, **no one** restores good land to the dispossessed, and **no one** alleviates the massively overcrowded living conditions.

Isolate any germ under the sun, give any medicine, **as long as** the fundamental horrendous facts of life remain the same, people will **die** in great numbers, and those in control will remain in control.

WHO & CFR: Globalise "humanitarian solutions"

The CFR is part of a sophisticated operation to globalise "humanitarian solutions" **under the rubric of medical care.** Its main ally is the World Health Organisation, an

agency of the UN. Near the close of World War II, **members** of the CFR were, in fact, tapped to write the basic outline of the **soon-to-be created** UN.

The WHO is on the march. It is trying to insert itself and its rulings and demands into the governments of many nations. In 2003, it won its **biggest** one-shot victory. **Through fraudulent travel advisories, based on non-science,** it raised fears about SARS (**at best, a tiny illness**) and managed to effectively **shut down air travel in and out of Toronto.** Toronto lost several billion dollars in the process. I was a peripheral part of a budding effort to convince local business owners to file a lawsuit. At first, there was some enthusiasm, but then it faded out. The people of Toronto knuckled under, some of them lost their shirts, and they plowed on.

The WHO is, **by far, the most successful agency of the** UN. It has emerged as the rising star of that moribund organisation. It has delivered **victories** because it is flying under the banner of medical power. **The modern priesthood.**

CFR, its **inner** core, is **well aware** that medical control is a trump card it can play to great advantage. The October Symposium was an event with such an edge.

This is **no** one-time takeover by force. This is no crashing

coup. In intelligence-agency parlance, it's a step-operation. A little progress here, a little progress there. Speakers at the Symposium called Swine Flu **"a teaching moment."** By this they meant **two** things. This **mild** flu gives CFR and its allies a chance to **expand** their **global** influence, through the expansion of "public"-health agencies, most notably WHO and the American CDC. And the population of the planet is **"taught" to respect so-called epidemics and** the resulting missives that come down from their leaders.

The pace of these **fake** epidemics and the accompanying media propaganda is quickening. There is an ultimate vision here that at least a few major power players entertain: subsume every citizen of planet Earth under a network of authoritarian medical control – as part of a **global**-management political system.

Cradle to grave, every person is diagnosed with at least several diseases or mental disorders and falls under the continuing treatment of doctors. These treatments are, for the most part, **toxic**. That is to say, they weaken the immune system and scramble neurotransmitter systems of the brain. People become less able to take effective action in any direction. People everywhere become fixated on their diseases. They become less able to maintain their freedom. They view themselves as lifelong patients.

Martinus Beijerinck and Ivanovski. The author credits these two men with the discovery of the virus. These are the men who took dying tobacco leaves, ground them up into a liquid, ran them through a filter, then injected the poison into the leaves of young growing plants and when the plants changed color, declared that something in the poison came **alive** and attacked the plants.

Do you know what they put in vaccines today? Worse than what they did years ago.

From A PARALYZING FEAR. 1998
The **so-called virus that they said caused polio.**

Page 183. " The flask broke and the virus spilled on the floor. We broke the 100-yard dash getting out of there. We came back and **Salk was mopping the floor, and he was laughing."**

And Fox News (2-6-2020) just reported the coronavirus had escaped from the lab in China.

What did Salk know that the other scientists didn't? That **this contagious virus is a myth.**

Attacking Those Who Don't Believe in the Coronavirus

Virus Mania http://www.whale.to/c/Virus-Mania55tt66.pdf
presents a tragic message that will, hopefully, contribute to the re-insertion of ethical values in the conduct of virus research, public health policies, media communications, and activities of the pharmaceutical companies.

Obviously, elementary ethical rules have been, to a very dangerous extent, neglected in many of these fields for an alarming number of years. When American journalist Celia Farber courageously published, in Harper's Magazine (March 2006) the article **"Out of control—AIDS and the corruption of medical science,"** some readers probably attempted to reassure themselves that this "corruption" was an isolated case. This is very far from the truth as **documented** so well in this book by Engelbrecht and Köhnlein. It is only the tip of the iceberg. Corruption of research is a widespread phenomenon currently found in many major, supposedly contagious health problems, ranging from AIDS to Hepatitis C, Bovine spongiform encephalopathy (BSE or "mad cow disease"), SARS, Avian flu and current vaccination practices (human papillomavirus or HPV vaccination).

In research on all of these **six** distinct public health concerns scientific research on viruses (or prions in the

case of BSE) slipped onto the wrong track following basically the same systematic pathway. This pathway always includes several key steps: **inventing** the risk of a disastrous epidemic, **incriminating** an elusive pathogen, **ignoring** alternative toxic causes, **manipulating epidemiology with non-verifiable numbers to maximize the false perception of an imminent catastrophe,** and **promising salvation with vaccines.**

This guarantees large financial returns. But how is it possible to achieve all of this? Simply by relying on the most powerful activator of human decision making process, i.e. **FEAR** !

We are not witnessing viral epidemics; we are witnessing epidemics of fear.

And both the media and the pharmaceutical industry carry most of the responsibility **for amplifying fears,** fears that happen, incidentally, to always ignite fantastically profitable business. Research **hypotheses** covering these areas of virus research are practically **never scientifically verified with appropriate controls.** Instead, **they are established by "consensus."** This is then rapidly reshaped into a dogma, efficiently perpetuated in a quasi-religious manner by the media, including ensuring that research **funding** is **restricted** to projects supporting the dogma, **excluding** research into alternative hypotheses. An important tool to keep dissenting voices out of the debate is **censorship** at various levels ranging from the

popular media to scientific publications. **We haven't learnt well from past experiences.** There are still many unanswered questions on the **causes** of the 1918 Spanish flu epidemic, **and** on the role of viruses in post-WWII polio (DDT neurotoxicity?). These modern epidemics should have opened our minds to more critical analyses. **Pasteur** and **Koch** had solidly constructed an understanding of infection applicable to many bacterial, contagious diseases. But this was **before the first viruses were actually discovered.** Transposing the principles of bacterial infections to **viruses** was, of course, very tempting but should **not** have been done without giving parallel attention to the innumerable risk factors in our **toxic** environment; to the **toxicity** of many drugs, and to some nutritional **deficiencies**. **Cancer** research had similar problems. The **hypothesis** that cancer might be caused by viruses was formulated in **1903**, **more than one century ago.** Even today it has **never** been convincingly demonstrated. Most of the experimental laboratory studies by virus-hunters have been based on the use of inbred mice, inbred implying a totally unnatural genetic background. Were these mice appropriate models for the study of human cancer? (we are far from being inbred!)

True, these mice made possible the isolation and purification of "RNA tumor viruses," later renamed "retroviruses" and well characterized by electron microscopy. But are these "viral" **particles** simply

associated with the murine tumors, or are they truly the culprit of malignant transformation? Are these **particles** real exogenous infective particles, or endogenous defective viruses hidden in our chromosomes? **The question is still debatable.** What is certain is that viral particles similar to those readily recognized in cancerous and leukemic mice **have never been seen nor isolated in human cancers.**

Of mice and men… However, by the time this became clear, in the late 1960s, **viral** oncology had achieved a dogmatic, quasi-religious status. If **viral** particles **cannot** be seen by electron microscopy in human cancers, the problem was with electron microscopy, **not** with the dogma of viral oncology! This was the time **molecular** biology was taking a totally dominant posture in viral research.
"Molecular markers" for **retroviruses** were therefore **invented** (reverse transcriptase for example) and **substituted most conveniently** for the **absent** viral particles, hopefully salvaging the central dogma of viral oncology. This permitted the viral hypothesis to survive for another **ten** years, until the late 1970s, with the help of increasingly generous support from **funding** agencies and from **pharmaceutical** companies. However by 1980 the **failure** of this line of research was becoming embarrassingly evident, and the **closing** of some viral oncology laboratories would have been inevitable, except

that… Except what? Virus cancer research would have crashed to a halt , **except** that, in 1981, **five cases of severe immune deficiencies were described by a Los Angeles physician, all among** homosexual men who were also all sniffing amyl nitrite, were all abusing other drugs, abusing antibiotics, and probably suffering from malnutrition and STDs (sexually transmitted diseases). It would have been logical to **hypothesize** that **these severe cases of immune deficiency had multiple toxic origins.** This would have amounted to incrimination of these patients' **life-style**… Unfortunately, such discrimination was, **politically**, totally unacceptable. **Therefore, another hypothesis had to be found**—these patients were suffering from a contagious disease **caused** by a **new…retrovirus!**

Scientific data in support of this hypothesis was and, amazingly enough, still is totally missing. That did not matter, and instantaneous and passionate interest of cancer **virus** researchers and institutions erupted immediately. This was salvation for the viral laboratories where AIDS now **became**, almost overnight, the main focus of research. It generated huge financial support from Big Pharma, more budget for the CDC and NIH, and **nobody** had to worry about the **life style** of the patients who became at once the innocent victims of this horrible virus, soon **labeled** as HIV.

Twenty-five years later, the HIV/AIDS hypothesis has **totally failed** to achieve three major goals in spite of the

huge research funding exclusively directed to projects based on it. No AIDS cure has ever been found; **no verifiable epidemiological** predictions have ever been made; and **no HIV vaccine** has ever been successfully prepared. Instead, **highly toxic (but not curative)** drugs have been most irresponsibly used, **with frequent, lethal side effects.** Yet **not** a single HIV particle has ever been observed by **electron** microscopy in the blood of patients supposedly having a **high viral load!** So what? All the most important newspapers and magazine have displayed **attractive computerized, colorful images of HIV** that all **originate** from laboratory cell cultures, **but never from even a single** AIDS patient. **Despite** this stunning omission the HIV/AIDS dogma is **still** solidly entrenched. Tens of thousands of researchers, and hundreds of major pharmaceutical companies continue to make huge profits based on the HIV **hypothesis**. And **not one** single AIDS patient has ever been cured… Yes, HIV/AIDS is emblematic of the corruption of virus research that is remarkably and tragically documented in this book. Research programs on Hepatitis C, BSE, SARS, Avian flu and current vaccination policies all developed along the same logic, that of maximizing **political control** & financial profits. Whenever we try to understand how some highly questionable therapeutic policies have been recommended at the highest levels of public health authorities (WHO, CDC, RKI etc.), we frequently discover either embarrassing **conflicts** of interests, or the

lack of essential control experiments, and always the **strict rejection of any open debate** with authoritative scientists presenting dissident views of the pathological processes. **Manipulations** of statistics, falsifications of clinical trials, dodging of drug toxicity tests have all been **repeatedly** documented. All have been swiftly covered up, and none have been able to, so far, disturb the cynical logic of today's virus research business. The cover-up of the neurotoxicity of the **mercury** containing preservative thimerosal as a highly probable cause of autism among vaccinated children apparently reached the highest levels of the US governement… (see article "Deadly Immunity" from Robert F. Kennedy Jr. in chapter 8)
Virus Mania is a social disease of our highly developed society. To cure it will require conquering fear, fear being the most deadly contagious virus, most efficiently transmitted by the media. to err is human, but to preserve an error is diabolic.

Etienne de Harven, MD Professor Emeritus of Pathology at the University of Toronto and Member of the **Sloan Kettering** Institute for Cancer Research, New York (1956 - 1981) Member of Thabo Mbeki's AIDS Advisory Panel of South Africa , President of Rethinking AIDS
(https://rethinkingaids.com
https://www.amazon.in/RETHINKING-AIDS-Rootbernst/dp/0029269059)

https://discoveriesrevealed.files.wordpress.com/2020/03/virus-mania.pdf

No virus of the H5N1 or H1N1 has been isolated
If no virus of the H5N1 been isolated, has ANY virus been isolated & shown to be able to reproduce?
An open letter to Professor **John Oxford, world renowned influenza virologist.**

I am writing to you because you appear to be the UK expert on viruses and particularly on pandemic viruses such as bird flu H5N1 or swine flu H1N1. I first came across you on the TV show "Pandemic" broadcast on the BBC on Tuesday 7th November 2006. Since then I have heard you on Irish and British radio on a number of occasions.

I have a simple question for you: **Can you name a scientific publication where a scientist has isolated, biochemically characterised and photographed the H1N1 virus or the H5N1 virus?**

If the H1N1 virus and the H5N1 virus actually do exist, you Professor Oxford should be able to either name a scientific publication proving their existence or isolate these viruses yourself using the techniques applied by Dr Lanka.

Kalpana Chauhan from the Customer Service Centre at the Department of Health in England provided me in July 2006 with some scientific papers which **allegedly** proved the existence of the mumps, measles and rubella viruses. Susanne Brix from Klein-Klein-Aktion examined these papers and **nowhere do the authors of these papers claim to have isolated these viruses.** This was pointed out to Kalpana Chauhan but she cut off our correspondence in August 2006.

These are the **eight** scientific papers provided by Kalpana Chauhan:

1.Immuno-Electron Microscopy of the Morphogenesis of Mumps Virus.
2.Hemadsorption of Mumps Virus Examined by Light and Electron Microscopy.
3.Electron microscopy of the development of rubella virus in BHK-21 cells.
4.Electron Microscopy of Monkey Kidney Cell Cultures Infected with RubellaVirus.
5.Antibody-induced capping of measles virus antigens on plasma membrane studied by electron microscopy.
6.Electron Microscopic Study on the Development of Measles Virus in Cultured Cells.
7.Fine Structure of Cellular Inclusions in Measles Virus Infections.

8.Observations of Measles Virus Infection of Cultured Human Cells: A study of Development and Spread of Virus Antigen by Means of Immunofluorescence.

These scientific papers **do not prove that the mumps, measles and rubella viruses actually exist.** Perhaps Professor Oxford you can examine them yourself and I believe you will agree that these papers **do not prove the existence of the mumps, measles and rubella viruses.**

Please do so.

I have published all this correspondence in my book **The Ultimate Conspiracy: The Biomedical Paradigm.** Professor Oxford I accept that it is your **opinion** and **that of your colleagues** that pathogenic viruses such as H1N1 and H5N1 exist. I am now well aware of your expert **opinion** from your numerous TV and radio broadcasts. What I am interested is the **primary evidence** on which any expert such as you should base his **opinion**.

As Lord **Nimmo Smith** said in an excellent judgment:

"But **however often a conclusion may be repeated, it is only as sound as the research on which it is based, and of this I have seen none."**

If this **primary evidence** is readily available, as it should

be, **if** the case for the existence of these pathogenic viruses is so self-evident, it should be possible for you Professor John Oxford to provide me and more importantly Dr **Stefan Lanka** with the relevant scientific publications. **If** you can**not** do so, then **you must publicly state why this is not possible.**

Why do we vaccinate against viruses that we cannot prove to exist.

However often Professor Oxford you proclaim about the **dangers of pandemics caused by fictitious viruses your opinions and** conclusions and assertions are only as strong as the research on which they are based.
Please produce **two primary references** that you can publicly defend, where the H5N1 virus and H1N1 virus have been isolated, biochemically characterised and photographed.

Yours Sincerely
James McCumiskey
Belfast, Ireland
http://www.proliberty.com/observer/20091243.htm

As of 8-29-19 I have put together **3** books on these subjects. Please scroll down to the bottom for information on ordering. This page with the **so-called polio virus** was

taken from a book written by **Alton Blakeslee** in 1956. Blakeslee was a press writer for the Associated Press. The book covered every detail on how Jonas Salk made his polio vaccine. Every detail **but one. The source of the so-called virus was not named.** See the source below under **Simon Flexner.** It was ground up spinal cord from a human child.

http://www.shotsoftruth.com/

Fake News
Virus History. 1898 T.M.V. 1954 Polio. 1957 Asian. 1961 Measles. 1976-1996 Swine Flu 2002 West Nile. 2004 Sars. 2005 Bird Flu. 2009 H1N1 2014 Ebola. 2016 Zika. 2018 Measles. 2019 Coronavirus.

Notice it says magnified 100,000 times with the electron microscope. The vaccine inserts **say** they have "**live'** virus in them. You can see that is a **lie**. The only real picture of a **so-called virus** is in black and white like the polio virus above. It will say that they are magnified up to 100,000 times. All **color pictures are computer generated or what is called an artist conception.** The fuzzy **white dots are not what you have been told--a live or dead agent of disease.** It is called in science , a pellet. It is the solid part of an infection , after being separated by a **centrifuge** machine.

"The electron microscope is capable of revealing details as much as 1000 times smaller than visible in light microscopes because the **wavelengths??** of electrons are much shorter than those of light. **Transmission** electron microscopes make it possible to explore cell structures and large protein molecules. Because beams of electrons**?? pass through** thin samples, cells and tissues

With **scanning** electron microscopes, a pencil like beam of electrons is scanned over the **surface** of a specimen. For images, specimens do **not** have to be cut into thin slices to be visualized. The scanning electron microscope produces scanning **three**-dimensional images of cells. Because electrons**??** are **easily scattered** by molecules in the **air**, samples examined in both types of electron microscopes must be placed in a **vacuum** in order to be studied. As a result, researchers **chemically preserve** their samples first and than **remove all the water** before placing them in the microscope.
…. THIS MEANS THAT ELECTRON MICROSOPY CAN BE USED TO VISUALIZE ONLY NON-LIVING PRESERVED CELLS AND TISSUES."

One doesn't have to be a scientist or doctor to understand the **way** an electron microscope works. Any intelligent 10 year old can understand the words, **THIS MEANS THAT ELECTRON MICROSCOPE CAN BE USED TO VISUALIZE ONLY NON-LIVING PRESERVED CELLS AND TISSUES."**

No one has EVER seen a live virus or antibody, because they don't exist. It is all a **scam** promoted by Thomas Rivers,M.D. in 1927. The word virus is from the Latin and means poison. Here is the **evidence** of what the virus really is and where it comes from. The virus that was promoted as the **cause** of polio and other diseases was just the poison or infection taken from a person or animal with a disease. The poison or infection was **not the cause of itself** -- it was the **result** of disease and therefore **cannot** be a prevention or cure of a disease.

THE REAL DEFINITION OF THE WORD VIRUS BEFORE IT WAS CHANGED BY THOSE WHO WANTED TO DECEIVE THE MEDICAL PROFESSION.

Webster's Shorter School Dictionary. 1927. virus, n. The poison of an infectious disease.

Thorndike Century Dictionary. 1935. 1. venom.2. A poison produced in a person suffering from a disease, as small-pox virus.

Webster's New Practical Dictionary. 1951. 1. venom. 2. The poison matter of a disease, as the virus of small-pox.

Webster's Encyclopedic Dictionary of the English language. 1957. virus. 1. venom. 2. A poison that is produced in the body by a disease.

"A poison that is produced in the body by a disease." The poison that is produced by the body in every disease is the infection or mucus. **The mucus or infection is the virus. The infection or the mucus is also the result of disease.** The infection or mucus **is what they make their vaccine out of.** So they make there vaccine **out of the result of disease not the cause of disease.**

This is **isopathy** which means the product of disease will prevent disease. Isopathy was coined in 1823 by Dr. Joesph Lux. Dr. Lux named it after Jenner who died in 1823. I would say it was a tribute **from one quack to another.**

let's think about this poison that the body produces in a diseased state. Using the **true** definition of the word virus, read that as small-pox pus. The pus is the poison produced by the body in the disease called small-pox.

Jonas Salk **said** vaccination is easy to understand. The vaccine is made from the virus that **caused** the disease. As you can see from the above **true** definitions Salk was deceptively referring to the infection of the disease. He was making his vaccine out of the **result** of disease, not the cause. He knew better. Edward **Jenner**, the man who is credited with the filthy practice of vaccination in the 1800s used the pus from the smallpox lesion and **said** if placed in the belly of a cow and then placed the cow pus

in the arm of a healthy person, that it would prevent smallpox.

Today all vaccines are still made from the result of a disease and claimed falsely to prevent disease. This is why the medical profession **hasn't** cured anybody in 2000 years. It is like saying a cancerous tumor is the cause of itself and then making a vaccine out of the cancer infection and claiming it will prevent cancer. Does that make sense, of course not, it is ridiculous. The real scientific term is not vaccination , nobody uses cow pus anymore, it's Isopathy which means the product of disease will prevent disease.

The **first and the only** question to ask yourself is , **if they couldn't see** the virus how did they know it existed?

From A PARALYZING FEAR. 1998

The so-called virus that they said caused polio.

Page 183. " The flask broke and the virus spilled on the floor. We broke the 100-yard dash getting out of there. We came back and Salk was mopping the floor, and he was **laughing**."

And Fox News (2-6-2020) just reported the coronavirus had escaped from the lab in China. LOL

A page from the 1947 Annual Report.
I will just write in a few more that were infiltrated by these shysters. Now you know why science is twisted.

California Institute of Technology 300,000.
New York Botanical Garden. 225,000.
University of Minnesota Medical School. 210,000.
Western Reserve University Clevland Ohio. 112,000
University of Pennsylvania. 111,300.
University of Chicago Ill. 25,800.
University of Pittsburgh. 32,000.
Stanford University San Francisco. 19,850.
University of Texas School of Medicine. 11,675.
And just about every college in the country.

THE VIRUS A HISTORY OF THE CONCEPT
(1977)

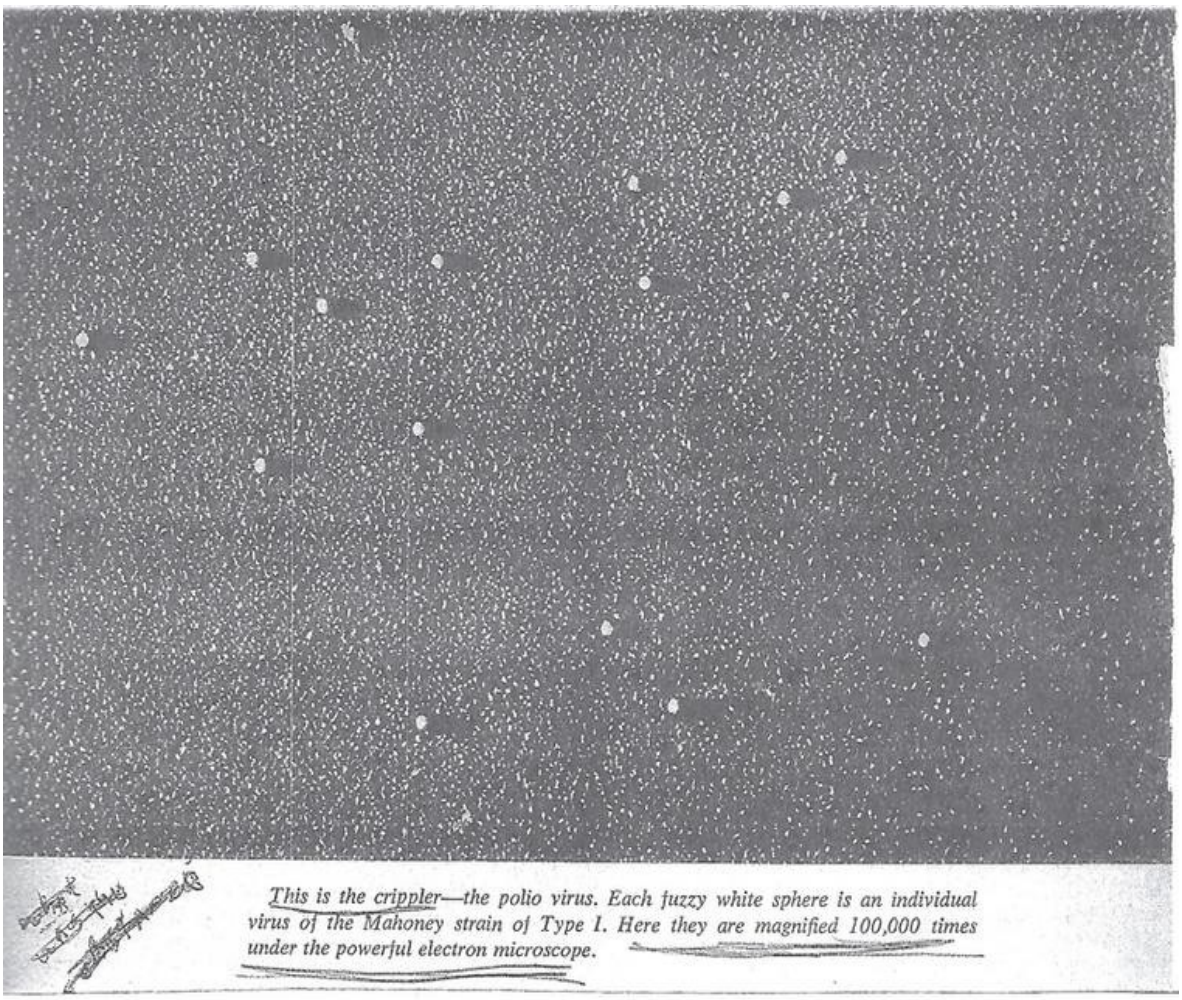
Preface

This history of the concept of the virus is written in nontechnical language for those with a general interest in science as well as for virologists and historians of science and medicine. Considering the clinical, theoretical and social importance of the viruses, it is surprising that so little of their history has appeared in print.*

Little emphasis has been placed in this book on dating the 'discovery' of the virus for two reasons. First, there is the problem of determining when it was discovered. Of the following, which should one designate? Its recognition as a filterable, submicroscopic infectious agent; its visualization in the light microscope as inclusion or elementary body; its subsequent visualization in the electron microscope; the elucidation of its molecular structure and function; or the enunciation of the earliest hypothesis approximating most closely its fundamental properties?

Second, the development of the present concept has been a cumulative one. Therefore, it would be misleading to point to one individual as the 'discoverer' of the virus, as has occurred in the case of Ivanovski and Beijerinck.

Others have credited these men with the 'discovery' of the



This is the crippler—the polio virus. Each fuzzy white sphere is an individual virus of the Mahoney strain of Type 1. Here they are magnified 100,000 times under the powerful electron microscope.

This picture of the virus said to cause polio was taken from the book,

"POLIO AND THE SALK VACCINE. WHAT YOU SHOULD KNOW ABOUT IT.

Written by Alton L. Blakeslee

Science editor, The Associated Press 1956

The author wants to acknowledge the close coperation which the National Foundation for Infantile Paralysis has given him at every stage in the development of this book. The author also expresses his appreciation to the following people for reading the book in galley proof and offering suggestions for correction: Dr. Jonas E. Salk; Dr. Albert B. Sabin; Dr Leonard Scheele, Surgeon-General of theeeee United States Health Service. Acknowledgment for photograph Eli Lilly, Indianapolis,Indiana.

Foreword by Basil O'Connor. President, the National Foundation for Infantile Paralysis

We at the National Foundatin for Infantile Paralysis have tried to keep the public informed. Newspapers, magazines, radio aaand television-have interpreted for the American people the findings of our scientists. Mr. Blakeslee knows his subject. We have a successful vaccine for polio. In the near future it can eliminate paralytic polio.

Once they had convinced the viruses were the **cause** of polio, then they would contact a university and ask if the university would accept so much money to set up a virus lab. Who could say no to the likes of Thomas Rivers?

And there is your "live virus." The pus of a smallpox sore. Poison.

From the book THE VIRUS: a History of the Concept. by Sally Smith Hughes. (1977)

Here she quotes Thomas Rivers the head of the virology department of the **Rockefeller** group and the head man in the Salk vaccine field trials of 1954.

He says let's just accept viruses are living.

And **Wendall Stanley**, also a **Rockefeller** stooge, who says that it may be **assumed** that viruses **require** the presence of living cells for reproduction.

All those tests were **color** tests. And the science is settled--When Pigs Fly.

Martinus Beijerinck called his **tobacco** leaf experiment a slimy liquid. Latin is a dead language, it doesn't change its

definition. Thomas Rivers in 1927 **began to change the meaning of virus to** today's B.S. definition.

That was in 1952. With all their medical advances it should be easy to find viruses in blood today. Try finding them.

In 1909 **Flexner** cut out the **spinal cord of a dead girl** and injected it into the **head** of a monkey.

In every 100 kids who didn't get vaccinated how many got polio?
And this condition of **malnutrition** was caused by what-a virus?

Monkeys did **not** get polio naturally. So they injected poison and looked for the **lesions** of polio. Lol

The book that exposes the Salk vaccine as a hoax.

The **foundation** of all vaccines given today is **based on these lies**.
1. Millions of people got polio in the 1950s.
2. Thousands died.
3. Polio was a disease of the central nervous system.
4. Polio was caused by viruses, living and dead.
All four beliefs are lies. You may not think that this is important, but remember that **all 50** vaccines given today

are **based on the lie** that the polio vaccine **wiped out** polio in the 1950s. It did not.

Viruses--Antibodies---Ghosts I also have CDs on the following subjects. 1. **The History of the Virus.** Part one. Time 33.12. 2. The History of the Virus. Part two. Time 25.19. 3. The History of the Virus. Part three. Time 27.56. 4. Tom Rivers,M.D. **Mis-educating the entire Medical Profession.** Time 42.08. 5. **The Selling of the Virus to the Public.** Part one. 25.29. 6. The Selling of the Virus to the Public. Part two. Time 17.40. 7. **The Salk Vaccine Cover-up of 1955.** Time 17.05. 8. **The Centrifuge and the Electron microscope and vaccine making.** Time 21.07. 9. **Herd** Immunity-Virus Shedding and other Myths. Time 42.35. 10. **Life of a Virus. Absolute proof of the Virus Lie.** Time 42.04. 11. Germs are not the Cause of Disease. Time 19.01. 12. Germ Theory by Hereward Carrington Time 42.13. 13. Germs are not the cause of the **Flu**. An experiment by a leading Dr. Absolute proof. Time 20.06. 14. Toxemia Explained by Dr. John Tilden. The **real** cause of disease. Time 73.15. 15. Disease is a Curing Process. Time 48.06. 16. **Why 50% of the kids died in the 1700s** Taken from a medical book written in 1793. Straight from the horses mouth. Time 17.06.

The Book of Health .. answers the following questions. 1. What is disease? 2. What is the cause of disease? 3.

Disease is a curing process. 4. The gem theory exploded. 5. How the organs of elimination become impaired. 6. The truth about fasting. 7. **The vitamin scam.** 8. **Biology terms hijacked by the chemist.** 9. **The syphilis Scam.** 10. **Rabies, does it even exist?** 11. **Leprosy, is not contagious.** 12. The living longer myth explained. 13. What is isopathy? 14. The truth about **Homeopathy**. 15. The truth about hand-washing and Semmelweis. 16. The truth about mitrochondria, and so much more.

book. The Virus- that never was.

They said the date April 12 was the 10 year anniversary of **Roosevelt's** birthday but was in reality 13 years to the day when Salk went to work for Thomas Francis at the University of Michigan.

The first and only filterable virus (poison) was the one Martinus Beijerinck made when he injected ground up tobacco leaves into fresh young plants and claimed that the poison had something in it that came alive and atack the plant.

O'Connor was a smart cookie. He brought in the biggest and best of businessmen into his group of supporters.

The reason the scientist concluded that vaccines could not work was that Broady and Park in 1935 vaccinated a

bunch of kids with the same vaccine that Salk used 20 years later and some of them died. At least they had enough sense to stop the program. And Thomas Rivers was right in the middle of that fiasco. Time makes people forget and Rivers knew that only to well.

Well, that should be able to be proved today with all the modern instruments. Find the circulating antibodies in the blood today.
It will **never** happen because it **didn't** happen. Yet **Salk never claimed his vaccine would cure polio.** He said **if** antibodies were created polio wouldn't happen. A great con.

The **Cutter** incident as it is called. What most people don't know is that **Louis P. Gebhardt** is the liar who claimed he found **live** virus in the Cutter vaccine. Gebhardt just covered for **Salk's** failure and deception. He was connected to the **Rockefeller** group.
Gebhardt worked with Salk on the typing program.

I have no idea **what** Smadel was talking about finding in the blood, but it sure **wasn't** circulating antibodies.

The real reason they brought them in was to bring in outside support so that if anything went wrong they would be covered, which plenty went wrong as you will see.

To see if it was safe. They told the media that it was as safe as safe could be.
Jim Shannon worked for the NIH. He questioned the vaccines. Due to pressure from the vaccine proponents, he was drowned out. He said the **only** safe vaccine ---was one that **never** was used.

Rivers was a key figure in this fraud.
Very freaking clever. If you get **one or** two shots and get polio they will just say that you were attacked by the **3rd** virus.

Tom Francis taught Jonas Salk **how** to make vaccines during WW 2 and Tom **Rivers** controlled everything and everybody.

Flexner injected ground-up spinal fluid from a child into the brain of a monkey, thus creating **artificial** disease.
The **monkey's** brain nerves were destroyed by the injection causing it to limp around.
The child **didn't** get polio by having anything injected into her brain.

There is that word **susceptible** again. You inject the poison in the animal, it gets sick or dies , you **claim** it was the germ, **not** the vaccine, and **if** it doesn't get sick you claim the vaccination worked. What a con job.

The **virus** of polio was the infection like Flexner ground up the spinal cord of that young girl who died of polio. So you can guess **what** the flu vaccine is made from. Snot. I call it the snot shot. **Making vaccines out of anything other than the infection of the disease would violate** the medical profession of the cause of disease and void making vaccines. So today if they used **other** ingredients it would be even a bigger scam.
And there you have it the saga of Jonas Salk. A liar, a cheat, a criminal. What was his motive? What were the motive of Rivers, O'Connor, and the rest of the gang of criminals?
Was it to make the children sick so as to secure customers for life for the medical profession?

Was it **depopulation**? Like **Bill Gates** is trying to do today. Salk wrote another book called the **Survival of the Wisest.** It should have been called the survival of the criminal mind. What went on in California that O'Connor **financed** for him. **Where did the money come from,** the American people? The Institute for Biological Studies in La Jolla California.

Salk is responsible for **all** the vaccines that are given today and causing many diseases, disability and death. And he prevented **no** disease and saved **no** lives. And the science is definitely **not** settled. And won't be until the truth comes out, which I fear will **never** happen as

evidenced bu the media spreading the **lies** about the Coronavirus today. (2020)

Status of COVID-19
As of 19 March 2020, COVID-19 is no longer considered to be a high consequence infectious diseases (HCID) in the UK. The 4 nations public health HCID group made an interim recommendation in **January** 2020 to classify COVID-19 **as** an HCID. This was based on consideration of the UK HCID criteria about the virus and the disease with information available during the **early** stages of the outbreak. Now that **more** is known about COVID-19, the public health bodies in the UK have reviewed the most up to date information about COVID-19 against the UK HCID criteria. They have determined that several features have now **changed**; in particular, more information is available about mortality rates (low overall), and there is now greater clinical awareness and a **specific and sensitive laboratory test?,** the availability of which continues to increase. The Advisory Committee on Dangerous Pathogens (ACDP) is also of the opinion that COVID-19 should **no** longer be classified as an HCID. The need to have a national, coordinated response remains, but this is being met by the government's COVID-19 response. Cases of COVID-19 are **no** longer managed by HCID treatment centres only. All healthcare workers managing possible and confirmed cases should **follow** the **updated** national infection and

prevention (IPC) guidance for COVID-19, which supersedes all previous IPC guidance for COVID-19. This guidance includes instructions about different personal protective equipment (PPE) ensembles that are appropriate for different clinical scenarios.

https://www.globalresearch.ca/secretary-state-mike-pompeo-admits-covid-19-live-exercise-president-trump-comments-i-wish-you-would-have-told-us/5707223
https://www.gov.uk/guidance/high-consequence-infectious-diseases-hcid

According to this UK document, as of 19 March 2020, COVID-19 is no longer considered to be a high consequence infectious disease (HCID) in the UK!! **So is there another reason for the lockdowns?**
Is this really a "Live Exercise" as mentioned by the US Secretary of State?

https://www.dailymail.co.uk/health/article-8143727/High-temperatures-humidity-significantly-slow-spread-coronavirus.html
https://www.gov.uk/guidance/high-consequence-infectious-diseases-hcid
How the British Government subjected thousands of people to chemical and biological warfare trials during Cold War 2015
https://www.independent.co.uk/news/uk/politics/how-the-

british-government-subjected-thousands-of-people-to-chemical-and-biological-warfare-trials-10376411.html
https://www.youtube.com/watch?v=eQLjqne2CEU
https://learninggnm.com/home.html
www.thesleuthjournal.com/bill-gates-population-control-microchip-2018/
https://www.rethink.org/news-and-stories/blogs/2020/03/coronavirus-temporary-changes-to-the-mental-health-act/
https://aim4truth.org/2020/03/18/dr-fauci-wants-to-kill-your-family/ https://www.youtube.com/watch?v=6PywRcxEhhI
https://www.youtube.com/watch?v=vL1pkARxr2U
https://www.youtube.com/channel/UCx4G-r9bVYg6xUID2YNbRaA/videos
https://www.youtube.com/watch?v=sUbLe9yUGGc

The Dream & Lie of Louis **Pasteur**.
Originally **Pasteur**: **Plagiarist**, **Imposter**. R.B. **Pearson**.
https://pdfs.semanticscholar.org/2c84/5f8dc8f2b191b001a8973a4e118cd2c7d909.pdf
http://www.whale.to/a/b/pearson.html

https://vexmansthoughts.files.wordpress.com/2017/04/bc3a9**champ-or-pasteur-a-lost-chapter-in-history-of-biology.**pdf

https://ia802800.us.archive.org/21/items/1.ThePoisonedNeedle/1.**thePoisonedNeedle**.pdf

Pasteur Exposed: The False Foundations of Modern Medicine Paperback – 1989 by Ethel Douglas Hume

James Hayton CORONAVIRUS
Lockdown: An Unnecessary Measure (With thanks to our medical advisers for the following information) As of 19 March 2020, COVID-19 is **no** longer considered to be a high consequence infectious disease (HCID) in the **UK**.

A new **French** study in the **Journal** of Antimicrobial Agents, titled SARS-CoV-2: fear versus data, concludes that "the problem of SARS-CoV-2 is probably **overestimated**“, since "the mortality rate for SARS-CoV-2 is **not** significantly different from that for common coronaviruses identified at the study hospital in France“.

An **Italian** study of August 2019 found that flu deaths in Italy were between 7,000 and 25,000 in recent years. This value is **higher** than in most other European countries due to the **large** elderly population in Italy, and much higher than anything , **attributed** to Covid-19 so far.

In a new fact sheet, the World Health Organization WHO reports that Covid-19 is in fact spreading **slower**, **not faster, than influenza by a factor of about 50%.**

Moreover, pre-symptomatic transmission appears to be much **lower** with Covid-19 than with influenza.

A leading **Italian** doctor reports that "strange cases of pneumonia“ were seen in the Lombardy region already in **November** 2019, raising again the question if they were caused by the new virus (which **officially** only appeared in Italy in **February** 2020), or by **other** factors, such as the dangerously high smog levels in Northern Italy.

Danish researcher Peter Gøtzsche, **founder** of the renowned Cochrane Medical Collaboration, writes that Corona is "an epidemic of mass panic“ and **"logic was one of the first victims.“**

Former **Israeli** Health Minister, Professor Yoram Lass, says that the **new** coronavirus is "**less** dangerous than the flu“ and lockdown measures "will kill more people than the virus“. He adds that „the numbers do not match the panic“ and "psychology is prevailing over science“. He also notes that **"Italy is known for its enormous morbidity in respiratory problems, more than three times any other European country.“**

Pietro Vernazza, a **Swiss** infectious disease specialist, argues that **many of the imposed measures are not based on science and should be reversed.** According to Vernazza, **mass** testing makes no sense because 90% of

the population will see no symptoms, and lockdowns and **closing** schools are even „counterproductive“.

The President of the World Doctors Federation, Frank Ulrich Montgomery, argues that lockdown measures as in Italy are „unreasonable“ and „counterproductive“ and should be **reversed**.

Switzerland: **Despite media panic, excess mortality still at or near zero:** the latest test-positive "victims“ were a 96 yo in palliative care and a 97 yo with pre-existing conditions. The **latest** statistical report of the Italian National Health Institute is now available in English. Above are today's statistics for COVID-19, including a graph showing the **weekly** numbers of deaths in the **UK** since 1 Jan 2020. As can be seen, the situation **so far shows a very similar track to the average of the past five years.**

https://www.eturbonews.com/568969/risk-of-dying-on-coronavirus/
https://www.globalresearch.ca/swiss-doctor-covid-19/5707642

The swine flu fraud of 1976, on 60 Minutes
https://www.youtube.com/watch?v=8elE7Ct1jWw

THIS IS NO COINCIDENCE!!!
https://www.youtube.com/watch?v=w--_b3VieXI
comparing with movie CONTAGION

"Under the right circumstances, people can be led to believe things that are not true.… On October 30, 1938, thousands of people **fled** from a crisis that had **no existence except in their imaginations.** A radio broadcast of H.G. Wells' "The War of the Worlds" led thousands of listeners to believe that the planet earth had been invaded by Martians! **We are ready to believe almost anything if** it comes from a recognized authority.

The world of Orwell's "1984" arrived unnoticed. Psychological warfare is being waged against an unsuspecting public through the control of mass media and the altering of public education. The population is being conditioned by a mass media-created culture. Long term exposure to this artificial reality cannot help but have an enormous impact on the social and political life of our nation. Add to this the inherent nature of television to induce the hypnotic state of mind regardless of content, and you have the most potent instrument for mass persuasion in the history of the world."

https://www.independent.co.uk/news/uk/politics/how-the-british-government-subjected-thousands-of-people-to-chemical-and-biological-warfare-trials-10376411.html

A Swiss Doctor on Covid-19

Published: March 14, 2020; **Updated**: March 25, 2020

A Swiss medical doctor provided the following information on the current situation.

According to the latest data of the Italian National Health Institute ISS, the average age of the positively-tested deceased in Italy is currently about 81 years. 10% of the deceased are over 90 years old. 90% of the deceased are over 70 years old.
80% of the deceased had suffered from two or more chronic diseases. 50% of the deceased had suffered from three or more chronic diseases. The chronic diseases include in particular cardiovascular problems, diabetes, respiratory problems and cancer.
Less than 1% of the deceased were healthy persons, i.e. persons without pre-existing chronic diseases. Only about 30% of the deceased are women.

The Italian Institute of Health moreover distinguishes between those who died from the coronavirus and those who died with the coronavirus. In many cases it is not yet clear whether the persons died from the virus or from their pre-existing chronic diseases or from a combination of both.
The two Italians deceased under 40 years of age (both 39 years old) were a cancer patient and a diabetes patient

with additional complications. In these cases, too, the exact cause of death was not yet clear (i.e. if from the virus or from their pre-existing diseases).
The partial overloading of the hospitals is due to the general rush of patients and the increased number of patients requiring special or intensive care. In particular, the aim is to stabilize respiratory function and, in severe cases, to provide anti-viral therapies.
(**Update**: The Italian National Institute of Health published a statistical report on test-positive patients and deceased, confirming the above data.)

The doctor also points out the following aspects:
Northern Italy has one of the oldest populations and the worst air quality in Europe, which has already led to an increased number of respiratory diseases and deaths in the past and is likely an additional risk factor in the current epidemic.
South Korea, for instance, has experienced a much milder course than Italy and has already passed the peak of the epidemic. In South Korea, only about 70 deaths with a positive test result have been reported so far. As in Italy, those affected were mostly high-risk patients.
The few dozen test-positive Swiss deaths so far were also high-risk patients with chronic diseases, an average age of more than 80 years and a maximum age of 97 years, whose exact cause of death, i.e. from the virus or from their pre-existing diseases, is not yet known.

Furthermore, according to a first Chinese study, the internationally used virus test kits may give a false positive result in some cases. In these cases, the persons may not have contracted the new coronavirus, but presumably one of the many existing human coronaviruses that are part of the annual (and currently ongoing) common cold and flu epidemics. (1)
Thus the most important indicator for judging the danger of the disease is not the frequently reported number of positively-tested persons and deaths, but the number of persons actually and unexpectedly developing or dying from pneumonia (**so-called excess mortality**).
According to all current data, for the healthy general population of school and working age, a mild to moderate course of the Covid-19 disease can be expected. Senior citizens and persons with existing chronic diseases should be protected. The medical capacities should be optimally prepared.
Medical literature
(1) Zhuang et al., Potential false-positive rate among the ‚asymptomatic infected individuals‘ in close contacts of COVID-19 patients, Chinese Medical Association Publishing House, March 2020.
(2) Grasselli et al., Critical Care Utilization for the COVID-19 Outbreak in Lombardy, JAMA, March 2020.
(3) WHO, Report of the WHO-China Joint Mission on Coronavirus Disease 2019, February 2020.
Reference values

Important reference values include the number of annual flu deaths, which is up to 8,000 in Italy and up to 60,000 in the US; normal overall mortality, which in Italy is up to 2,000 deaths per day; and the average number of pneumonia cases per year, which in Italy is over 120,000. Current all-cause mortality in Europe and in Italy is still normal or even below-average. Any excess mortality due to Covid-19 should become visible in the European monitoring charts.

Updates
March 17, 2020 (I)
The mortality profile remains **puzzling** from a virological point of view because, in contrast to influenza viruses, children are spared and men are affected about twice as often as women. On the other hand, this profile **corresponds** to natural mortality, which is close to **zero** for children and almost twice as high for 75-year-old men as for women of the same age.
The younger test-positive deceased almost always had severe pre-existing conditions. For example, a 21-year-old Spanish soccer coach had died test-positive, making international headlines. However, the doctors diagnosed an **unrecognized leukemia**, whose **typical** complications include severe pneumonia.

The decisive factor in assessing the danger of the disease is therefore **not** the number of test-positive persons and

deceased, which is often mentioned in the media, but the number of people actually and unexpectedly developing or dying from pneumonia (so-called excess mortality). So far, this value remains very low in most countries.
In Switzerland, some emergency units are already overloaded simply because of the large number of people who want to be tested. This points to an additional psychological and logistical component of the current situation.

March 17, 2020 (II)
Italian immunology professor Sergio Romagnani from the University of Florence comes to the conclusion in a study on 3000 people that 50 to 75% of the test-positive people of all ages remain completely symptom-free – significantly more than previously assumed.
The occupancy rate of the North Italian ICUs in the **winter** months is typically already 85 to 90%. Some or many of these existing patients could also be test-positive by now. However, the number of additional unexpected pneumonia cases is **not** yet known.

A hospital doctor in the Spanish city of Malaga writes on Twitter that people are currently **more likely to die from panic and systemic collapse than f**rom the virus. The hospital is being overrun by people with colds, flu and possibly Covid19 and **doctors have lost control.**

March 18, 2020
A new epidemiological study (preprint) concludes that the fatality of Covid19 even in the **Chinese city of Wuhan was only 0.04% to 0.12% and thus rather lower than that of seasonal flu, which has a mortality rate of about 0.1%.** As a reason for the **overestimated** fatality of Covid19, the researchers suspect that **initially** only a small number of cases were recorded in Wuhan, as the disease was probably asymptomatic or mild in many people.
Chinese researchers argue that extreme **winter** smog in the city of Wuhan may have played a **causal** role in the outbreak of pneumonia. In the summer of 2019, public **protests** were already taking place in Wuhan because of the **poor** air quality.
Northern Italy has the highest levels of air pollution in Europe, and how this air pollution has been greatly reduced by the quarantine.

A manufacturer of the Covid19 test kit states that it should only be used for research purposes and not for diagnostic applications, as it has not yet been clinically validated.

March 19, 2020 (I)
The Italian National Health Institute ISS has published **a new report on test-positive deaths:**

The median age is 80.5 years (79.5 for men, 83.7 for women).
10% of the deceased was over 90 years old; 90% of the deceased was over 70 years old.
At most 0.8% of the deceased had no pre-existing chronic illnesses.
Approximately 75% of the deceased had two or more pre-existing conditions, 50% had three more pre-existing conditions, in particular heart disease, diabetes and cancer.
Five of the deceased were between 31 and 39 years old, all of them with serious pre-existing health conditions (e.g. cancer or heart disease).
The National Health Institute hasn't yet determined what the patients examined ultimately died of and refers to them in general terms as Covid19-positive deaths.
March 19, 2020 (II)
A report in the Italian newspaper Corriere della Sera points out that Italian intensive care units already collapsed under the marked flu wave in 2017/2018. They had to postpone operations, call nurses back from holiday and ran out of blood donations.
German virologist Hendrik Streeck argues that Covid19 **is unlikely to increase total mortality in Germany,** which normally is around 2500 people per day. Streeck mentions the case of a 78-year-old man with preconditions who died of heart failure, subsequently tested positive for

Covid19 and thus was included in the statistics of Covid19 deaths.
According to Stanford Professor John Ioannidis, argues that there is **no** reliable medical data backing the measures **currently** decided upon.

March 20, 2020
According to the latest European monitoring report, overall mortality in all countries (including Italy) and in **all age groups remains within or even below the normal range so far.**
According to the latest German statistics, the median age of test-positive deaths is about 83 years, most with pre-existing health conditions that might be a possible cause of death.

March 21, 2020 (I)
Spain reports only three test-positive deaths under the age of 65 (out of a total of about 1000). Their pre-existing health conditions and actual cause of death are not yet known.
On March 20, Italy reported 627 nationwide test-positive deaths in one day. By comparison, normal overall mortality in Italy is about 1800 deaths per day. Since February 21, Italy has reported about 4000 test-positive deaths. Normal overall mortality during this time frame is up to 50,000 deaths. **It is not yet known to what extent normal overall mortality has increased, or to what**

extent it has simply turned test-positive. Moreover, Italy and Europe have had a very mild flu season in 2019/2020 that has spared many otherwise vulnerable people.
According to Italian news reports, **90% of test-positive deceased in the Lombardy region have died outside of intensive care units,** mostly at home or in general care sections. Their **cause of death and the possible role of quarantine measures in their deaths remain unclear.**

Only 260 out of 2168 test-positive persons have died in ICUs.

Bloomberg highlights that „99% of Those Who Died From Virus Had Other Illness, Italy Says“

March 21, 2020 (II)
The Japan Times asks: **Japan was expecting a coronavirus explosion. Where is it?** **Despite** being one of the first countries getting positive test results and having imposed no lockdown, Japan is one of the **least**-affected nations. Quote: „Even if Japan may not be counting all those infected, hospitals aren’t being stretched thin and there has been **no** spike in pneumonia cases.“
Italian researchers argue that the **extreme** smog in Northern Italy, the worst in Europe, may be playing a

causative role in the current pneumonia outbreak there, as in Wuhan before.
In a new interview, Professor Sucharit Bhakdi, a world renowned expert in medical microbiology, says blaming the new coronavirus alone for deaths is „**wrong**“ and „**dangerously misleading“,** as there are **other more important factors at play**, notably pre-existing health conditions and poor air quality in Chinese and Northern Italian cities. Professor Bhakdi describes **the currently discussed or imposed measures as „grotesque“, „useless“, „self-destructive“ and a „collective suicide“** that will shorten the lifespan of the elderly and should **not** be accepted by society.

March 22, 2020 (I)
Regarding the situation in Italy:
Most major media falsely report that Italy has up to 800 deaths per day from the coronavirus. In reality, the president of the Italian Civil Protection Service stresses that these are deaths „with the coronavirus and not from the coronavirus“ (minute 03:30 of the press conference). In other words, these persons died while also testing positive.

As Professors Ioannidis and Bhakdi have shown, countries like **South Korea and Japan that introduced no lockdown measures have experienced near-zero excess mortality in** connection with Covid-19,

while the Diamond Princess cruise ship experienced an **extrapolated** mortality figure in the per mille range, i.e. **at or below the level of the seasonal flu.**

Current test-positive **death figures in Italy are still less than 50% of normal daily overall mortality in Italy,** which is around 1800 deaths per day. Thus it is possible, perhaps even **likely**, that a large part of normal daily mortality **now simply counts as** „Covid19“ deaths (as they test positive). This is the point stressed by the President of the Italian Civil Protection Service.
However, by now it is clear that **certain regions in Northern Italy, i.e.** those facing the **toughest lockdown measures, are experiencing markedly increased daily mortality figures.** It is also **known** that in the Lombardy region, 90% of test-positive deaths occur **not** in intensive care units, but **instead** mostly at home. And more than 99% have **serious pre-existing** health conditions.

Professor Sucharit Bhakdi has called lockdown measures „useless“, „self-destructive“ and a „collective suicide“. Thus the extremely troubling question arises **as to what extent the** increased mortality of these elderly, isolated, **highly stressed** people with multiple pre-existing health conditions may in fact be **caused** by the weeks-long lockdown measures still in force.
If so, it may be one of those cases where **the treatment is worse than the disease.** (See update below: **only 12% of**

death certificates show the coronavirus as a cause. --- even there the tests done are not scientific or reliable)

Angelo Borrelli, head of the Italian Civil Protection Service, emphasizing the **difference** between deaths with and from coronaviruses.

March 22, 2020 (II)
In Switzerland, there are currently 56 test-positive deaths, all of whom were **„high risk patients“** due to their advanced age and/or pre-existing health conditions. Their actual cause of death, i.e. from or simply with the virus, has **not** been communicated.
The Swiss government claimed that the situation in southern Switzerland (next to Italy) is „**dramatic**“, yet local doctors **denied** this and said **everything is normal.**
According to press reports, oxygen bottles may become scarce. The reason, however, is not a currently higher usage, but rather **hoarding** due to fear of future shortages.
In many countries, there is already an increasing shortage of doctors and nurses. This is primarily because healthcare workers testing positive have to self-quarantine, even though in many cases they will remain fully or largely symptom-**free**.

March 22, 2020 (III)

A model from Imperial College London predicted between 250,000 and 500,000 deaths in the UK „from“ Covid-19, but the authors of the study have now **conceded** that many of these deaths would **not** be in addition to, but rather **part** of the normal annual mortality rate, which in the UK is about 600,000 people per year. In other words, **excess mortality would remain low.**

Dr. David Katz, founding director of the Yale University Prevention Research Center, asks in the New York Times: **„Is Our Fight Against Coronavirus Worse Than the Disease?** There may be more targeted ways to beat the pandemic.“

According to Italian Professor Walter Ricciardi, **„only 12% of death certificates have shown a direct causality from coronavirus“**, whereas in public reports „all the people who die in hospitals **with** the coronavirus? are deemed to be dying **of** the coronavirus“. This means that Italian death figures reported by the media have to be **reduced** by at least a factor of 8 to obtain actual deaths caused by the virus. Thus one ends up with at most a few dozen deaths per day, compared to an overall daily mortality of 1800 deaths and up to 20,000 flu deaths per year.

March 23, 2020 (I)

A **new French** study in the Journal of Antimicrobial Agents, titled SARS-CoV-2: fear versus data, concludes that „**the problem of SARS-CoV-2 is probably overestimated“,** since „the mortality rate for SARS-CoV-2 is not significantly different from that for common coronaviruses identified at the study hospital in France“.

An Italian study of August 2019 found that flu deaths in Italy were between 7,000 and 25,000 in recent years. This value is **higher** than in most other European countries due to the large elderly population in Italy, and **much higher than anything attributed to** Covid-19 so far.

Danish researcher Peter Gøtzsche, founder of the renowned Cochrane Medical Collaboration, writes that Corona is „an epidemic of mass panic“ and „logic was one of the first victims.“

March 24, 2020
The UK has **removed** Covid19 from the official list of High Consquence Infectious Diseases (HCID), stating that mortality rates are **„low overall“.**
The director of the German National Health Institute (RKI) admitted that **they count all test-positive deaths, irrespective of the actual cause of death, as „coronavirus deaths“.** The average age of the deceased is 82 years, most with **serious** preconditions. As in most

other countries, excess mortality due Covid19 is likely to be near **zero** in Germany.

Beds in Swiss intensive care units reserved for Covid19 patients are **still „mostly empty“.**

German Professor Karin Moelling, former Chair of Medical Virology at the University of Zurich, stated in an interview that Covid19 is „**no killer virus“ and that „panic must end“.**

In Italy, overall national mortality of the 65+ age group until March 7 remained below the level of earlier years, especially due to the rather **mild** winter (see red line in chart below).

March 25, 2020
German immunologist and toxicologist, Professor Stefan Hockertz, explains in a radio interview that Covid19 is no more dangerous than influenza (the flu), but that it is simply observed much more closely. More dangerous than the virus is the **fear** and panic created by the media and the „authoritarian reaction“ of many governments. Professor Hockertz also notes that most so-called „corona deaths“ have **in fact died of other causes while also testing positive for coronaviruses.** Hockertz believes that up to **ten** times more people than reported already had Covid19 **but** noticed nothing or very little.

The Argentinean virologist and biochemist Pablo Goldschmidt explains that Covid19 is no more dangerous than a bad cold or the flu. It is even possible that the Covid19 virus circulated already in earlier years, but wasn't discovered because no one was looking for it. Dr. Goldschmidt speaks of a **„global terror" created by the media and politics. Every year, he says, three million newborns worldwide and 50,000 adults in the US alone die of pneumonia.**

Professor Martin Exner, head of the Institute for Hygiene at the University of Bonn, explains in an interview why health personnel are currently under pressure, even though there has **hardly been any increase in the number of patients in Germany so far.**

Professor Julian Nida-Ruemelin, former German Minister of State for Culture and Professor of Ethics, points out that Covid19 poses no risk to the healthy general population and that **extreme measures such as curfews are therefore not justified.**

Using data from the cruise ship Diamond Princess, Stanford Professor John Ioannidis showed that **the age-corrected** lethality of Covid19 is between 0.025% and 0.625%, i.e. in the range of a strong cold or the flu. Moreover, a Japanese study showed that of all the test-positive passengers, and despite the high average age,

48% remained completely symptom-free; even among the 80-89 year olds 48% remained symptom-free, while among the 70 to 79 year olds it was an astounding 60% that developed no symptoms at all. This again raises the question whether the pre-existing diseases are not perhaps a **more important factor** than the virus itself. The Italian example has shown that 99% of test-positive deaths had one or more pre-existing conditions, and even among these, only 12% of the death certificates mentioned Covid19 as a causal factor.

On Corona, the Media, and Propaganda
19 March 2020;
In the current situation, **the old and proven** propaganda rule applies again: **the less is known, the more is speculated.**
For attentive readers, however, this offers an opportunity to assess the standards and focus of different media outlets and authors.

One may ask, for example:
Who merely counts test-positive case and death figures **without** asking what these people actually fall ill with or die of?
Who brings **headlines** such as , 21-year-old football coach dies of coronavirus“ and **only mentions in the last sentence that he had undiagnosed leukaemia**?

Who addresses the issue of so-called **excess** mortality, which is **still within or even below** the normal range in all countries and age groups?
Who asks how many **additional**, unexpected pneumonia patients there are in intensive care units, and what their age and health profile is?
Who prefers **frightening** pictures of viruses, protective suits and coffins rather than **actual** data, facts and background information?
Who discusses the well-known problems with **virus test kits** in general, and the **missing clinical validation** of the currently used virus test kit in particular?
Who highlights the problematic role played by the WHO in previous cases, and in this one?
Who is trying to add a **political or geopolitical spin** to the current situation?
Who is *still* talking about , **biological weapons**", even though this scenario has long been **ruled out** by hardly spectacular death rates and death profiles?
The bioweapons **rumor**, which has been launched on **every** occasion for almost forty years, **primarily** serves a geopolitical and psychological purpose. (See also: **History of Biological Warfare**)

Medical and military experts asked by SPR recommend keeping three possible scenarios in mind when analyzing current developments („the three P's"):
A **pandemic** of a dangerous virus

A media-induced **mass psychosis**
A potential **psychological operation**

As an example of recent psychological operations, they mention the repeatedly **staged** chemical weapons attacks in the **Syria** war, which have been **uncovered??** since 2019 by whistleblowers of the OPCW and other experts, but have been largely ignored by the mass media.

Here's what the CDC says about the test for the Coronavirus Mar 24 , 2020 by Jon Rappoport

Straight from the horse's mouth—both sides
The CDC (US Centers for Disease Control) **admits the coronavirus test is flawed.** That's the overview and the takeaway— As my readers know, I've described why the widespread diagnostic **test** for the coronavirus is insufficient, **misleading, useless, and deceptive.** That test, **used all over the world** where it is available, is called the PCR. It DIAGNOSES patients. "Yes, you have the virus." "No you don't." A very alert reader sent me a link to a US Centers for Disease Control (CDC) document about the test. **The CDC establishes the guidelines for how the test should be done, and what the results mean.** Here is a CDC paragraph about results. I suggest you read it several times. **"Positive**

[test] results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities."

I'm going to blow past the blatant **contradiction** in that CDC paragraph and cut to the chase. The **key** line in that paragraph is: "The agent detected [the coronavirus] may **not** be the definite **cause** of disease."
… the test could say the coronavirus is there in somebody's body, **but the virus may not be causing disease…** On one level, the CDC is admitting the test could turn up **false** positives: the test could say a patient **has** the coronavirus, but he really **doesn't**. This **isn't** a footnote stuck at the bottom of a report. It's right there near the top of the section about the **meaning** of the test. On a deeper level, the CDC is saying straight out,
IF THE TEST SHOWS A CORONAVIRUS IS PRESENT, THAT DOESN'T MEAN IT'S CAUSING DISEASE.

The test has an inherent problem. **At best,** it might show that a virus is present in the patient's body. But the test is incapable of determining **how much virus is actively replicating in the patient's body.** And why is that important? Because, to even begin to say a **virus** is

causing actual illness in a human, **there would have to be millions and millions of a virus replicating in his body** and the PCR test has **never been proven, in the real world, to be able to make such a judgment accurately.** But, if you read that CDC quote again, you'll see the CDC is ordering labs **to report** a positive test result to public health agencies where it will be counted as a "coronavirus case".

https://www.cdc.gov/coronavirus/2019-ncov/lab/index.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Flab%2Frt-pcr-detection-instructions.html

Is this test FDA-approved or cleared? No. This test is not yet approved or cleared by the United States FDA. When there are **no** FDA-approved or cleared tests available, and other **criteria??** are met, FDA **can?** make tests available under an emergency access mechanism called an Emergency Use Authorization (EUA). The EUA for this test is supported by the Secretary of Health and Human Service's (HHS's) declaration that circumstances exist to **justify??** the emergency use of in vitro diagnostics for the detection and/or diagnosis of COVID-19. This EUA will remain in effect (meaning this test can be used) for the duration of the COVID-19 declaration justifying emergency use of IVDs, **unless** it is terminated

or revoked by FDA (**after which the test may no longer be used**). **There is limited information available about the spectrum of illness associated with COVID-19 but it likely**?? spreads to others when a person shows signs or symptoms of being sick (e.g., fever, coughing, difficulty breathing, etc.). However, it is possible for this test to give a negative result that is incorrect (**false** negative) in some people with COVID-19. This means that you could possibly still have COVID-19 even though the test is negative. If this is the case, your healthcare **provider** will consider the test result together with your symptoms, possible exposures, and geographical location of places you have recently traveled) in deciding how to care for you. (interpretation / judgement / **arbitrary**).

Why was my sample tested? You were tested because your healthcare provider **believes?** you may have been exposed to the virus that causes COVID-19 **based on your signs and symptoms** (e.g., fever, cough, difficulty breathing), and/**or** because: • You live in or have recently traveled to a place where transmission of COVID-19 is known to occur, and/or • You have been in close contact with an individual suspected of or confirmed to have COVID-19. Your samples will **help??** find out if you have COVID-19.

What are the **known and potential risks** and benefits of the test? Potential **risks** include: • Possible discomfort or

other complications that can happen during sample collection. • **Possible incorrect test result** (see below for more information). Potential benefits include: • The results, along with other information, can help your healthcare provider make informed**??** recommendations about your care. • The results of this test may help limit the spread**??** of COVID-19 to your family and others in your community. What does it mean **if** I have a positive test result? If you have a positive test result, it is very **likely** that you have COVID-19. Therefore, it is also **likely** that you may be placed in isolation to avoid spreading the virus to others. There is a very **small?** chance that this test can give a positive result that is **wrong** (a false positive result). Your healthcare provider will work with you to determine**??** how best to care for you based on the test results, medical history, and your symptoms.
https://www.cdc.gov/coronavirus/2019-ncov/downloads/Factsheet-for-Patients-2019-nCoV.pdf

The virus causing COVID-19 is called SARS-CoV-2. It is **thought** to spread mainly from person-to-person via respiratory droplets among close contacts. // Recent studies indicate that people who are infected but do not have symptoms **likely** also play a role in the spread of COVID-19. https://www.cdc.gov/coronavirus/2019-ncov/community/guidance-law-enforcement.html

April 4th ... COVID-19 is a new disease and **we are still learning about how it spreads and the severity of illness it causes.!!!!!!!!!!** The virus is **thought** to spread mainly from person-to-person. .. Some recent studies have suggested that COVID-19 **may** be spread by people who are not showing symptoms. .. It **may** be possible that a person can get COVID-19 by touching a surface or object that has the virus on it and then touching their own mouth, nose, or possibly their eyes. This is not **thought** to be the main way the virus spreads, **but we are still learning more about this virus.** ... Information from the ongoing COVID-19 pandemic **suggest** that this virus is spreading **more** efficiently than influenza, but **not** as efficiently as measles, which is **highly** contagious**???**. .. COVID-19 is **thought** to spread mainly through close contact from person-to-person in respiratory droplets from someone who is infected. People who are infected often have symptoms of illness. Some people without symptoms **may** be able to spread virus. ... https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html

The virus that causes COVID-19 is spreading from person-to-person. People are **thought** to be most contagious when they are symptomatic (the sickest). That is why CDC recommends that these patients be isolated either in the hospital or at home (depending on how sick they are) until they are better and no longer pose a risk of

infecting others. More recently the virus has also been detected in asymptomatic persons. **How long someone is actively sick can vary so the decision on when to release someone from isolation is made using a test-based or non-test-based strategy** (i.e. time since illness started and time since recovery) **in consultation with state and local public health officials.** The decision involves considering the specifics of each situation, including disease severity, illness signs and symptoms, and the results of laboratory testing for that patient. Learn more about CDC's guidance on **when to release** someone from isolation and discharge hospitalized patients with COVID-19. For information on when someone who has been sick with COVID-19 is able to stop home isolation see Interim Guidance for Discontinuation of In-Home Isolation for Patients with COVID-19. Someone who has been **released** from isolation is **not** considered to pose a risk of infection to others. **There is much more to learn about the transmissibility, severity, and other features associated with COVID-19 and investigations are ongoing.**
https://www.cdc.gov/coronavirus/2019-ncov/faq.html

There are ongoing investigations to learn more.

A: As stated in Section IV.D of the FDA's *Policy for Diagnostic Tests for Coronavirus Disease-2019*, the FDA

does not intend to object to the development and distribution by commercial manufacturers, or development and use by laboratories, of serology tests to identify antibodies to SARS-CoV-2, where the test has been validated, notification is provided to FDA, and information along the lines of the following is included in the test reports:

This test has not been reviewed by the FDA.

Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a **molecular** diagnostic should be considered to rule out infection in these individuals.

Results from **antibody** testing should **not be used as the sole basis to diagnose or exclude** SARS-CoV-2 infection or to inform infection status.

Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

This policy does **not** apply to **at home** testing.

The commercial manufacturers and laboratories listed below have notified FDA that they have validated and are offering serology tests as set forth in Section IV.D of the FDA's *Policy for Diagnostic Tests for Coronavirus Disease-2019*. **The FDA has not reviewed the validation of tests offered by these developers,** who will not be pursuing EUAs, and is including this list here to

provide transparency regarding the notifications submitted to FDA.
https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2

These sequences are intended to be used for the purposes of respiratory virus surveillance **and research.** The recipient agrees to use them in compliance with all applicable laws and regulations. Every effort has been made to assure the accuracy of the sequences, **but CDC cannot provide any warranty regarding their accuracy.** The recipient can acknowledge the source of sequences in any oral presentations or written publications concerning the **research** project by referring to the Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA.
2019-N
https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.
Acceptable Alternative Primer and Probe Sets.
The following lots of N1, N2, and RP primers and probes have **passed functional** testing at CDC and may be used with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel Instructions for Use **under** CDC's Emergency Use

Authorization (EUA): https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations Please note that **only** the listed lot numbers of material are acceptable for testing under CDC's EUA. Any primer and probe reagents included in these kits in **addition** to N1, N2 and RP have **not** been tested by CDC and **may not be used for diagnostic testing under** CDC's EUA. Only N1, N2 and RP primer and probe sets may be used. https://www.cdc.gov/coronavirus/2019-ncov/downloads/List-of-Acceptable-Commercial-Primers-Probes.pdf

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in upper and lower respiratory specimens during infection. Positive results are **indicative** of active infection with 2019-nCoV but do **not** rule out bacterial infection or co-infection with other viruses. **The agent detected may not be the definite cause of disease.** Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. Negative results do **not** preclude 2019-nCoV infection and should **not** be used as the sole basis for treatment or other patient management decisions. Negative results must be **combined** with clinical observations, patient history, and epidemiological information. Testing with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is intended for use by **trained** laboratory personnel who are

proficient in performing real-time RT-PCR assays. The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is **only for use under** a Food and Drug Administration's Emergency Use Authorization.

The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is a molecular in vitro diagnostic test that aids in the detection and diagnosis 2019-nCoV and **is based on widely used nucleic acid amplification technology.** The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) and control material used in rRT-PCR for the in vitro **qualitative** detection of 2019-nCoV RNA in respiratory specimens. The term "qualified laboratories" refers to laboratories in which **all** users, analysts, and any person reporting results from use of this device should be **trained** to perform and **interpret** the results from this procedure by a competent instructor prior to use.

RNA isolated and purified from upper and lower respiratory specimens is reverse transcribed to cDNA and subsequently **amplified** in the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 **software**. In the process, the probe **anneals** to a specific target sequence located between the **forward and reverse primers.** During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase

degrades the probe, causing the reporter **dye** to separate from the quencher dye, generating a **fluorescent** signal. With each cycle, additional reporter dye **molecules** are **cleaved** from their respective probes, increasing the fluorescence intensity. **Fluorescence intensity is monitored at each** PCR cycle by Applied Biosystems 7500 Fast Dx Real-Time PCR System with SDS version 1.4 **software**. Detection of viral RNA not only **aids** in the diagnosis of illness but **also** provides epidemiological and surveillance information.

Only material distributed through the CDC International Reagent Resource and specific lots of material posted to the CDC website are acceptable for use with this assay under CDC's Emergency Use Authorization.

Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time **reagents** used in the amplification step become **contaminated** by accidental introduction of amplification product (amplicon).

Do not refreeze probes. Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use. Training in specimen collection is

highly recommended due to the importance of specimen quality.

The nCoVPC consists of in vitro transcribed RNA. The nCoVPC will yield a positive result with the following primer and probe sets: N1, N2 and RP. Human Specimen Control (HSC) (Extraction Control) When HSC is run with the CDC 2019-nCoV rRT-PCR Diagnostic Panel (see previous section on Assay Set Up), the HSC is used as an RNA extraction procedural control **to** demonstrate successful recovery of RNA as well as extraction reagent integrity. The HSC **control** consists of **non**infectious**??** cultured human cell (A549) material. Purified nucleic acid from the HSC should yield a positive result with the RP primer and probe set and negative results with all 2019-nCoV markers. **Expected** Performance of Controls Included in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel Control Type External Control Name Used to Monitor 2019 nCoV_N1 2019 nCoV_N2 RP **Expected** Ct Values Positive nCoVPC

If the RP assay does not produce a positive result for human clinical specimens, **interpret** as follows:¬ − If the 2019-nCoV N1 and N2are positive **even** in the absence of a positive RP, the result should be **considered** valid. It is possible, that some samples may **fail** to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does **not** preclude

the presence of 2019-nCoV virus RNA in a clinical specimen. − If all 2019-nCoV markers AND RNase P are negative for the specimen,the result should be **considered invalid** for the specimen. If residual specimen is available, **repeat** the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a **new** specimen should be collected if possible. 2019-nCoV Markers (N1 and N2) • When all controls exhibit the expected performance, a specimen is considered negative if **all** 2019- nCoV marker (N1, N2) cycle threshold growth curves DO NOT cross the threshold line within 40.00 cycles (< 40.00 Ct) AND the RNase P growth curve DOES cross the threshold line within 40.00 cycles (< 40.00 Ct). • When all controls exhibit the expected performance, a specimen is **considered** positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct). The RNase P may **or may not be** positive as described above, but the 2019-nCoV result is **still** valid. • When all controls exhibit the expected performance and the growth curves for the 2019-nCoV markers (N1, N2) AND the RNase P marker DO NOT cross the cycle threshold growth curve within 40.00 cycles (< 40.00 Ct), the result is **invalid**. The extracted RNA from the specimen should be **retested**. If residual RNA is not available, **re**-extract RNA from residual specimen and re-test. If the retested sample is negative for all markers and RNase P, the result is invalid

and collection of a new specimen from the patient should be considered. • When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 but not both markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, reextract RNA from residual specimen and re-test. If the same result is obtained, report the inconclusive result. Consult with your state public health laboratory or CDC, as appropriate, to request guidance and/or to coordinate transfer of the specimen for additional analysis. • **If** HSC is positive for N1 or N2, then **contamination** may have occurred during extraction or sample processing. Invalidate all results for specimens extracted alongside the HSC. Re-extract specimens and HSC and re-test.

35 CDC-006-00019, Revision: 03 CDC/DDID/NCIRD/ Division of Viral Diseases Effective: 3/30/2020 2019-nCoV rRT-PCR Diagnostic Panel Results **Interpretation** Guide The table below lists the expected results for the 2019-nCoV rRT-PCR Diagnostic Panel. If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and **cannot** be resolved through the recommended re-testing, please contact CDC for consultation and possible specimen referral.

Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have **not** been determined. Collection of **multiple** specimens (types and time points) from the same patient may be necessary to detect the virus. • A **false** negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification **inhibitors** are present in the specimen or if **inadequate** numbers of organisms are present in the specimen. • Positive and negative predictive values are **highly dependent on prevalence.** False negative test results are more likely when **prevalence of disease is high.** False positive test results are more likely when **prevalence is moderate to low.** • If the virus **mutates** in the rRT-PCR target region, 2019-nCoV may **not** be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. **An interference study evaluating the effect of common cold medications was not performed.** • Test performance can be affected because the epidemiology and clinical spectrum of infection caused by 2019-nCoV is **not fully known.** For example, clinicians and laboratories may **not** know the optimum types of specimens to collect, and, during the course of infection, when these specimens are **most** likely to contain levels of viral RNA that can be readily detected. • **Detection of viral RNA may not indicate the presence of infectious**

virus or that 2019-nCoV is the causative agent for clinical symptoms.

• The performance of this test has not been established for monitoring treatment of 2019-nCoV infection. • The performance of this test has not been established for screening of blood or blood products for the presence of 2019-nCoV. • This test cannot rule out diseases caused by other bacterial or viral pathogens.

Probe sequence of 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed **no** sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is **no significant homologies with human genome,** other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

2019-nCoV_N2 Assay: The forward primer sequence of 2019-nCoV rRT-PCR assay N2 showed high sequence homology to Bat SARS like coronaviruses. The reverse primer and probe sequences showed **no** significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is **no** prediction of potential false positive rRT-PCR results.

In summary, the 2019-nCoV rRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed **no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict** potential false positive rRT-PCR results.
https://www.fda.gov/media/134922/download

FACT SHEET FOR HEALTHCARE PROVIDERS CDC –

2019-nCoV Real-Time RT-PCR Diagnostic Panel
Updated: March 15, 2020

Coronavirus Disease 2019 (COVID-19)
This Fact Sheet informs you of the **significant known and potential risks** and benefits of the emergency use of the Centers for Disease Control and Prevention (CDC) 2019-nCoV Real-Time RT-PCR Diagnostic Panel.
Testing is to be conducted on specimens from people who meet Coronavirus Disease 2019 (COVID-19) clinical and/or epidemiological **criteria** for testing. What are the symptoms of COVID-19? **Many** patients with confirmed COVID-19 have developed fever and/or symptoms of acute respiratory illness (e.g., cough, difficulty breathing). However, **limited information is currently available to characterize the full spectrum of clinical illness associated with COVID-19. Based on what is known about the virus that causes** COVID-19,

signs and symptoms may appear any time from 2 to 14 days after exposure to the virus. Based on preliminary data, the median incubation period is approximately 5 days but may range 2-14 days. Public health officials have **identified?** cases of COVID-19 throughout the world, including in the United States.

• The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel can be used to test upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, bronchoalveolar lavage, sputum, lower respiratory tract aspirate, nasopharyngeal wash/aspirate or nasal aspirate). • The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel should be ordered for the detection of the virus that causes COVID-19 in individuals who meet the COVID-19 clinical and/or epidemiological criteria for testing. • The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is authorized for use in laboratories in the United States, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform **high complexity tests** Specimens should be collected with appropriate infection **control precautions.**

Laboratory test results should always be considered in the context of clinical observations and epidemiological data in making a final diagnosis and patient management decisions.

This test is to be performed **only** using respiratory specimens collected from individuals who meet COVID-19 clinical and/or epidemiological criteria for testing.

In the event of a **false** positive result, **risks** to patients could include the following: a recommendation for isolation of the patient, monitoring of household or other close contacts for symptoms, patient isolation that might limit contact with family or friends and **may increase contact with other potentially COVID-19 patients,** limits in the ability to work, **the delayed diagnosis and treatment for the true infection causing the symptoms, unnecessary prescription of a treatment or therapy, or other unintended adverse effects.**

A negative test result for this test means that SARSCoV-2 RNA was not present in the specimen above the limit of detection. However, a negative result does not rule out COVID-19 and **should not be used as the sole basis for treatment or patient management decisions.** A negative result does not exclude the possibility of COVID-19. When diagnostic testing is negative, the possibility of a false negative result should be considered in the context of a patient's recent exposures and the presence of clinical signs and symptoms consistent with COVID-19. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical

presentation indicate that COVID19 is likely, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If COVID-19 is still suspected based on exposure history together with other clinical findings, re-testing should be considered by healthcare providers in consultation with public health authorities. **Risks** to a patient of a false negative include: delayed or lack of supportive treatment, lack of monitoring of infected individuals and their household or other close contacts for symptoms resulting in increased risk of spread of COVID-19 within the community, or other unintended adverse events.

What is an EUA? The United States FDA has made this test available under an **emergency** access mechanism called an Emergency Use Authorization (EUA). The EUA is supported by the Secretary of Health and Human Service's (HHS's) declaration that circumstances exist to justify the emergency use of in vitro diagnostics (IVDs) for the detection and/or diagnosis of COVID-19. An IVD made available under an EUA **has not undergone the** same type of review as an FDA-approved or cleared IVD. FDA may issue an EUA when certain criteria are met, which includes that there are no adequate, approved, available alternatives, and based on the totality of scientific evidence available, it is reasonable to **believe** that this IVD may be effective in the detection of the virus that causes COVID-19. The EUA for this test is in effect

for the duration of the COVID-19 declaration justifying emergency use of IVDs, unless terminated or revoked (after which the test may no longer be used). https://www.cdc.gov/coronavirus/2019-ncov/downloads/Factsheet-for-Healthcare-Providers-2019-nCoV.pdf

Damn! Will the Zombie Virus Apocalypse never come? by **Jon Rappoport**. March 2, 2020 .

A correct reading of suppressed medical history reveals that the **hypothesis** of "one disease, one germ" is a modern **con** , moving down a blind alley at midnight. And when you **add** "one vaccine" to the formula, you get an even greater degree of lunacy. But you also get a trillion-dollar commercial success. I don't care how many contemporary molecular biologists are working in labs, amplifying **invisible** slivers **of who knows what** molecules into view, **and calling them viruses;** it's a **con**. This also applies to biowar biologists trying to create super-germs. They're all working in the dark vis-à-vis the natural processes of the body, which are far more complex and far more protective of health than these scientists know—unless the body is interfered with by direct poisons or gross mechanical destruction. The history of human health shows that upgrades in public **sanitation**, hygiene, and improved nutrition have done more for people than all the "germ-fighting"

pharmaceutical interventions ever invented laid end to end. But THAT is not a trillion-dollar commercial success. Even when tissue samples are taken from the body, properly separated through **centrifuge**, and then observed under an electron microscope, by the most competent and honest researchers, **you still get dead pictures of dead particles.** As researcher **Karma Singh** has pointed out, you **don't** know, **from those pictures, what such particles do or don't do when they're alive and integrated in the body. You can't infer that they cause disease.** The whole operation of the Virus Hunters is one brassy late-night infomercial tap dancing in the long, long history of humans on this planet. You want germs? No one knows how many there are. From various estimates, we could be talking about thousands of trillions to the thousandth power. Maybe more. If an infinitesimal fraction of the critters caused serious disease, we'd not only all be dead, we'd be dead on dead on dead. To begin to understand how **overblown** all these modern epidemic duds are, let's go to the animals. Farm animals. Pigs. A headline blares: **A MILLION PIGS SLAUGHTERED.** African Swine Fever **Virus** was discovered, and in order to stop the contagion, death was rained down on the pigs. On the farm. On the giant factory farm. So a question arises: Do you seriously think humans sat down next to each of the million pigs and tested him/her for the Virus? Drew a blood or tissue sample? **Twenty** pigs tested positive and they killed the rest as matter of course. They always do.

But wait. What are the conditions on this massive million-pig factory farm? Let's see. Pigs living in their own urine and feces, crowded next to one another, nose to butt, sprayed with **toxic** chemicals, **eating** chemical-laced feed —under high **stress**, **never** living the kind of existence they were **designed** for. Think they're going to get sick? Think some kind of minimally **reliable** test might find a virus or two living and replicating in their bodies? Do you seriously think those **viruses** matter, contrasted against the **obvious** immunosuppressive environment?
The researchers are using **indirect** methods of virus detection (PCR, antibody tests), and as a result, they have **no** idea what they're discovering. It could be fragments of random "DNA or RNA", cellular debris, germs that live quite comfortably in the body and **never** cause harm.

How are viruses discovered and identified in the first place?
The earthshaking Etienne De Harven interview by Celia Farber by Jon Rappoport February 18, 2020
The question I've been asking since **1987**— If the experts are going to claim a particular virus **causes** a particular disease, **how do they know that virus exists in the first place?** For example, the supposedly new coronavirus in China. For example, Ebola. For example, HIV. For example, the coronavirus supposedly causing SARS (2003). **How do researchers know these viruses exist?**

When scientists tell us they're rushing to develop a vaccine against a virus that is harming the population, **how do they know that virus exists to begin with?**

They're talking about lockdowns and quarantines **without** having proved their favorite virus of the moment exists.

Etienne De Harven. The interview was conducted several years ago by the brilliant reporter, Celia Farber.

- Serious Adverse Events: An Uncensored History of AIDS ..2006 by Celia Farber

  http://www.robertogiraldo.com/eng/papers/Farber_Reply_April_2006.html
  http://www.papelesdesociedad.info/IMG/pdf/rethinking_aids.pdf

Etienne De Harven's background: **president of the Electron Microscopy Society of America;** researcher, Memorial Sloan-Kettering Cancer Center; Cornell professor of cell biology; professor of pathology, University of Toronto; recognized pioneer in the field of electron microscopy.

The interview focuses on HIV; whether it was ever found and isolated. **The implications and questions spread out to any and all viruses.**

DE HARVEN: Unacceptably frustrated by the total lack of success in all attempts to demonstrate virus particles in human cancer by EM, the "impresarios" of the cancer/virus "dream" (**Gallo**, **Fauci**, and others) totally engaged in the molecular approach. Consequently, they invented molecular markers to compensate for the **missing** viral particles…This would have been acceptable **if** the specificity of these new molecular markers would have been clearly established. Unfortunately, this was not the case. The **most misleading** molecular marker was probably the **first** one, i.e. the **enzyme [called] reverse transcriptase (RT).** Following Temin and Baltimore 1970 papers in "Science", the RT enzymatic **activity** has been, most **abusively**, used as a **specific** retroviral marker. Both Temin and Baltimore demonstrated RT activity in samples of **supposedly** "**purified**" **retrovirus**. Embarrassingly, they **both omitted** to verify the "purity" of their samples by EM. Some of their samples were simply **purchased** from a commercial company… True, the **label** on the vials read "pure retrovirus"… However, it was known that these commercial "pure retrovirus" were **heavily** contaminated by cellular debris! And since it is **also** known that **all** cells contain RT (see Varmus), cellular debris are most **likely** carrying similar RT enzymes. Temin and Baltimore did **not**, therefore, **prove** that RT is a specific molecular marker for retroviruses. It would have been so simple**?** to check, by EM, the degree

of "purity" of the samples they used. This would have, most probably, shown important cell **debris** contamination, and would have obliged Temin and Baltimore to be much more cautious in the **interpretation** of their results. In 1975, the members of the **Nobel** Committee, most regrettably, **failed** to scrutinize this "purity" problem… In 1983, at Pasteur Institute in Paris, reliance on the **RT** marker **was a key element in the claimed "isolation" of a new retrovirus** [HIV].

Still, **Montagnier** himself recognized **"We did not purify"**… He dangerously **omitted** to consider the misleading **interference** of cell debris, just as Temin and Baltimore did in 1970. But a paper on the discovery of a **new retrovirus** looks much better if it contains at least… one EM picture! So, members of Montagnier's team spent **hours** at the TEM [transmission electron microscope], looking at their **mixed** cell cultures, and they **found the virus!** See Fig. 2 in their "historic" 1983 "Science" paper! It is, by the way, a good quality EM picture. It shows unquestionable retroviral particles, budding at the surface of a cell. **But the legend of** this Fig. 2 states that this cell **is a cord blood lymphocyte.** Indeed, cord blood lymphocytes were **admixed** to these complex cell cultures **(why?)**.

Montagnier and his co-workers should have known that human embryonic tissues, and the placenta in particular, are very rich in **endo**genous **retroviruses** (HERVs), and

that cord blood lymphocytes should **therefore** be expected to carry the same endogenous retroviruses (**under the TEM, endogenous and exogenous viruses, looking identical, cannot be distinguished.**) The budding of these particles has perhaps been **stimulated** by some of the **growth factors also present in** these cell cultures. An essential **control** would have been to repeat the experiment using lymphocytes from the peripheral blood **instead** of from cord blood. This control is unfortunately **missing**. In short, I would frankly state that the **Pasteur** 1983 paper (whose 30th anniversary has just been celebrated in a "grand messe" of **official** HIV retro-virology!) contributed **very little** in AIDS research because its conclusion (i.e. **"the isolation of a new retrovirus"**) is **based** on 1) the use of a **non** specific RT molecular marker, and 2) is **falsely** supported by EM pictures of, most **probably**, **endo**genous human retroviruses. More details and appropriate references on this analysis can be found in my 2010 paper published in the Journal of American Physicians and Surgeons [— "Human Endogenous Retroviruses and AIDS Research: Confusion, Consensus, or Science?"] (https://www.jpands.org/vol15no3/deharven.pdf).

CELIA FARBER: When antibody and VL [**viral load**] tests became widespread as diagnostic tools for "HIV infection" over the ensuing decades, what happened with EM inside of HIV science and literature? It is my

understanding that **nobody** has ever found HIV in human blood, on EM. Is this an accurate way to say it?

DE HARVEN: In my views, **Western Blot [antibody] tests lost all credibility after** the publication of Eleni Papadopulos's et al. (1993) paper, and **antibody** tests ("**Elisa**") [lost credibility] after Christine Johnson's report (1996).

The **notion** of a "Viral load" (VL), however, brought a new parameter in AIDS diagnosis (Ho,1996). It called attention to the **actual** number of HIV particles **supposedly** present in the blood plasma of AIDS patients, PCR technologies [tests] being **presumed** to offer a way to **quantify** that number. **If** such a viremia (i.e. presence of virus particles in the blood) is **indeed** present in AIDS patients, it reminisces the retroviral viremia **well known in** leukemic mice. In **such** case, **retroviral** particles should be readily demonstrable, by TEM, of appropriately prepared patient plasma samples. Unfortunately, it has **never** been possible to demonstrate by TEM , **one** single retroviral particle in the blood plasma of **any** AIDS patient, **even if** one selects patients presenting with a so-called "**high** viral load." I was apparently the **first** researcher to make that statement, during the opening session of President T. **Mbeki's** major AIDS conference, in Pretoria, SA, in May 2000. My statement to that effect has **never** been refuted.

CELIA FARBER: How come?

DE HARVEN: That question must be answered because **"something" is measured by** PCR technologies in the blood of many AIDS patients. **Actually, what is being measured is definitely not the number of retroviral particles** (phantom-like, i.e. EM invisible!). In fact, what is being PCR identified, amplified, and supposedly quantified is the number of genomic nucleotide sequences that are extremely similar to sequences known to be part of the retroviral genome. Most regrettably, these sequences were **misinterpreted** as an indication as a certain number of … HIV particles! This did a lot to consolidate the quasi-religious dogma of HIV as the cause of AIDS, a dogma that has been sharply criticized, a few years ago, by **David Rasnick who wrote, authoritatively, about "The AIDS Blunder**"… This **interpretation** would have been acceptable **only if** retroviral particles would have been readily demonstrated, by EM, in the blood plasma of these patients; but, since this is not the case, **another** explanation for the presence of these nucleotide sequences has to be founded. I presented at the RA conference in Oakland, CA, in 2009, and further developed in my 2010 JAPS paper such a much needed explanation for the presence of these retroviral-like nucleotide sequences. My explanation is based on the well known, variable amounts of circulating

DNA in the blood of severely ill patients, and on the fact that we all carry [**irrelevant**] retroviral-like sequences in our DNA, as **endo**genous, defective retroviruses, i.e. HERVs (HERVs, for "Human endogenous retroviruses") (See "Virus in all of us", R. Lower at al., 1996 PNAS paper). No surprise, therefore, that these nucleotide sequences are **recognized by PCR** [tests] in the blood of many AIDS patients, who are indeed severely ill. As already demonstrated in 2008 in **Robin Weiss laboratory,** HERVs can **interfere** as confounding factors in the search for novel retrovirus in chronic human diseases…

CELIA FARBER: …Paint a picture for us. The story of the [HIV] virus, the **"new deadly virus,"** what happens first: What steps did they [—] Montagnier, on one hand, **Gallo** on the other [—] **take to "find"** the new entity? Then once they 'found' it, what shape was it in? It was not an entity, a thing, with a body, right? It was not coherent. Can we say that? So it lived where? **It was seen only through the technologies developed to find it,** Elisa, WB [both are antibody tests]? **Later** PCR/VL [tests]? But what happened back then when they tried to see it on EM? Why **didn't** everybody look for it on EM? Too expensive?

DE HARVEN: No, EM is not cheap but not that expensive! And its cost has certainly nothing to do with

the fact that it has **barely been used for the past** 30 years in AIDS research! It has **not** been used because "They" knew it was **not** going to show anything of retroviral significance in samples coming directly from AIDS patients. And since AIDS had become big business, the stocks of involved giant pharmaceutical companies could not be jeopardized! It had to be saved at all cost, even at the cost of trusting **non specific** molecular markers… **Fear is good business, and viruses generate fear most efficiently**… So, the HIV flag has to be maximally agitated. **In worldwide medias, with thousands of computer-generated, colorful caricatures of an idealistic retrovirus**… By contrast, the medias have been dominated by **the most rigorous censorship** when it comes to inform the public about views of rethinking dissidents. This total censorship put a safety lock on any information that could jeopardize the colossal, entirely HIV derived profits of the major pharmaceutical companies. But I am glad we have Internet! Daring to say that HIV does **not** exist amounts to some sort of a capitalistic crime… Yes, the HIV dogma is probably the **darkest** page in the history of modern medicine.

CELIA FARBER: Etienne, if you could sum up: **Does HIV exist?** If so, where and how and as what? If you could examine 1,000 HIV positive people's blood under EM, what would you expect to find? If you **don't** find

HIV on EM in human blood, can any argument be made that the virus is “hiding” and so forth, or that the drugs suppressed the virus to undetectable levels? This is what the defenders of the orthodoxy seem to be saying about the results seen in the Nushawn Williams case.

DE HARVEN: This is the main question! Questioning the very existence of HIV is not something that should be debated only between specialized retro-virologists. It is an essential question that concerns all of us.

CELIA FARBER: Why?

DE HARVEN: Simply because 100% of AIDS research funding is based on the dogmatically postulated existence of HIV. If HIV does not exist, it would follow that AIDS research is the most appalling case of total misappropriation of public research funds! And it would also follow that the monumental amounts of money, so far exclusively devoted to HIV research, would be much better used in other directions. Could you imagine what world we would live in, today, if the total amount of money wasted over the past 30 years on HIV research had been, instead, used for feeding starving Africans, for clean water supply equipment, for public hygiene infrastructures, and for public health education? This would happen only if HIV research is totally stopped! And for this, the scientific and public health organizations

have to face **the fact that, indeed, HIV does not exist!** …we all have to, courageously, face the fact that the very existence of an **exo**genous HIV **has never been scientifically verified.**

https://www.davidrasnick.com/aids/aids-blunder.html
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4172096/
https://www.ias.ac.in/article/fulltext/jbsc/028/04/0383-0412
https://www.virusmyth.com/aids/index/drasnick.htm
https://www.modernghana.com/news/903640/hivaids-greatest-medical-fraud-of-21st-century.html

How many other unproven viruses have likewise been prematurely massaged into existence and prominence? How many times have researchers pulled "special markers" like rabbits out of hats—spuriously claiming these markers establish the existence of otherwise never-observed viruses? And therefore, **when these researchers state they have published the genetic sequences of these viruses—what are they really sequencing?**

And when someone steps forward, and **claims a new and never-before-seen virus is actually a** manmade weapon, and he knows this from studying its genetic sequence, **is he right, or** is he looking at the sequence of an irrelevant microbe that has been rudely coaxed from its long languishing snooze in the warmth of the human body?

ABSTRACT Human Endogenous Retroviruses (HERVs) are confounding factors in HIV/AIDS research that cannot be ignored. Evidence suggests that “viral load” may actually be measuring retroviral nucleoside sequences associated with HERVs. HERVs also provide a **valid** explanation for the presence of retroviruses recognizable by electron microscopy (EM) in the original 1983 publication from the Institut Pasteur, and may account for claims of innumerable “mutations” of the putative HIV pathogen. The interference of HERVs in AIDS research **brings into question** the subject of study in so-called “AIDS Research,” and **the very existence of an** exogenous HIV pathogen itself.

The HIV **Consensus!!!!!!!**

The **hypothesis** that the acquired immunodeficiency syndrome (AIDS) is caused by an exogenous retrovirus, the human immunodeficiency virus (HIV), initially proposed in the early 1980s, has exclusively dominated AIDS research for the past 25 years, although many investigators have **repeatedly stressed the lack of scientifically acceptable verification of this hypothesis.** Alerted to the numerous **shortcomings** of the official retroviral hypothesis by eminent retrovirologist Peter Duesberg, a group of AIDS “Rethinkers,” founded by molecular biologist **Charles Thomas** in 1991, called for

the “Scientific Reappraisal of the HIV/AIDS Hypothesis” in 1996. This group (www.rethinkingaids.com) released a mission statement co-signed by **thousands** of scientists and concerned **citizens**, including Nobel laureates **Walter Gilbert** and **Kary Mullis.** Other well-respected scientists, notably Sonnabend, Stewart, Lang, Papadopulos, Rasnick, and Geshekter and distinguished scientific writers such as Celia Farber, John Lauritsen, Neville Hodgkinson, Joan Shenton, Christine Maggiore, Renaud Russeil, Djamel Tahi, Jean-Claude Roussez, and Janine Roberts have also described the multiple failings of the HIV hypothesis. Between 1992 and 2000, another group based in London, UK, made highly significant contributions to scientific/public education by publishing magazine, under the leadership of Huw Christie. A medical team directed by Eleni Papadopulos in Perth, Australia, has also presented information **questioning** the validity of the HIV hypothesis. In May 2000, the controversy concerning HIV and the “antiretroviral” (ARV) drugs used to treat it became the topic of international inquiry when President Thabo Mbeki of South Africa, convened a debate between 35 academic scientists, “Orthodoxers” as well as “Rethinkers” together. A similar debate took place in 2003 at the Continuum European Parliament in Brussels, Belgium, when Paul Lannoye, a Belgian member of parliament, organized a public debate on “AIDS in Africa.” Reports by AIDS Rethinkers are readily accessible on numerous websites, the early and most

significant ones being www.virusmyth.com , www.rethinkingaids.com , www.theperthgroup.com , www.sidasante.com , and www.altheal.org . In spite of innumerable scientific and public conferences and publications by AIDS Rethinkers, many in the medical community either ignore, or bluntly reject the existence of any HIV controversy, or claim that AIDS "denialism" undermines AIDS prevention. As a result, the monumental budgets allocated throughout the world to combat AIDS have been, and still are totally and exclusively restricted to HIV research. This can **neither** be explained nor justified by the lack of alternative hypotheses of AIDS **causation**, since **nonviral** factors (chemical, pharmacological, nutritional, and behavioral) associated with the clinical symptoms attributed to AIDS have been well **documented** and reviewed by others. The retroviral hypothesis linking HIV to AIDS received a precipitous acceptance, **not** on the basis of scientifically verifiable data, **but** based on a so-called "**consensus**"—a consensus enthusiastically supported by the **pharmaceutical** industry. This review will focus primarily on the scientific facts (or artifacts) that impact the credibility ofAIDS research. In the extensive HIV/AIDS literature, one finds that the **claimed** "evidence" that AIDS is caused by HIV-1 or HIV-2 is presumably "clear-cut, exhaustive and unambiguous," and comprises **four** groups of data: (1) identification of retroviral molecular markers, (2) observation of retroviral

particles by transmission EM, (3) claimed efficacy of antiretroviral (ARV) drugs, and (4) epidemiological data.

In a long list of **presumed** HIV molecular markers, the **most** emblematic one **is the enzyme** reverse transcriptase (RT). **Importantly**, however, the activity of this enzyme has been readily demonstrated in practically **all** living "cells" of the biological universe, making it **imperative** to verify the **purification** of "viral" samples , **before** making any claim for a specific **link** between RT **and** retroviruses.

Sample **contamination** by "cell" debris can, by itself, explain the presence of RT activity. This is of considerable **importance** because attempts to **isolate** and **purify** HIV by **sucrose gradient ultracentrifugation** of **supernatant** from **supposedly** HIV infected cell cultures have provided samples **heavily contaminated** with microvesicular "cell" debris,readily demonstrated**??** by EM.

Factors that gave apparent **credibility** to the HIV Hypothesis.

(1). Identification of Retroviral Molecular Markers
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Human Endogenous Retroviruses and AIDS Research: Confusion, Consensus, or Science? Journal of American Physicians and Surgeons Volume 15 Number 3 Fall 2010

Anti-HIV **anti**bodies are regarded as another class of molecular markers, used in so-called "HIV tests," such as the **enzyme**-linked immunosorbent assay (ELISA). The **lack** of specificity of this test, however, was clearly documented by C. Johnson who reported, as early as **1996**, that almost **70** medical conditions having **nothing** to do with AIDS or HIV may **result in a positive antibody test.** These conditions **include** tuberculosis, malaria, leprosy, hepatitis, blood transfusions, influenza vaccination, multiple pregnancies, and others. **Such a lack of specificity came as no surprise** to those who were aware that the method used to prepare "HIV" **antibodies** was **based on a circular argument,** as discussed early on by **Neville Hodgkinson.** Moreover, the **method initially** used in ELISA tests included a **400-fold plasma dilution. Without** such high dilution **everybody** turned out to be "HIV positive,"as **originally** demonstrated by **Roberto Giraldo** in 1998.
Protein antigens of **claimed** retroviral origin represent a group of HIV markers used in another "HIV test," the western blot test (WB). The WB test is **used , to** confirm the ELISA test, and is **based on** the identification by electrophoresis on polyacrylamide gels of 10 **presumably** HIV proteins,**??** such as p120, p41, p32, p24/25, and

others. However, **prior** successful isolation and purification of HIV would be required to verify that all of these proteins **actually** originate from HIV particles, a purification that has **never** been achieved, as **recognized** by Luc Montagnier himself. The considerable difficulty in isolating **and** purifying HIV was recognized, as early as 1993, by Eleni Papadopulos et al., who correctly concluded that **without** successful HIV purification, the **retroviral nature** of the "HIV marker proteins" was **most un**certain. Papadopulos emphasized that these proteins are **most likely** cellular, originating from the abundance of cell **debris** in **poorly "purified"** HIV samples. The uncertainty **and** shortcomings of WB testing were already reported in 1991. Soon afterwards, Papadopulos et al. raised the question: **"Is a positive western blot , proof of HIV infection?"** That WB tests are **not reliable is evidenced by** , the **variability** of the protein criteria required for a "positive" test , in **different countries**. **The test is not even approved for diagnostic purposes in Great Britain.** The considerable difficulties experienced in attempts to **purify** "HIV" have **never** been resolved. Recently, **Henry Bauer** has reviewed evidence that supports the conclusion that **"HIV tests are not HIV tests."** "HIV tests" only indicate the presence of antibodies **supposedly** directed against HIV. They do **not** indicate the presence of the virus itself. The question then arises of whether the so-called **"viral load"** tests are more reliable, as they are **based on** polymerase chain reaction

(PCR) technologies for recognizing and quantifying HIV. This appears **highly questionable;** Nobel laureate Kary Mullis himself, the discoverer of PCR, has indicated that his method is **not expected to provide a reliable result** in HIV diagnosis. A **second** reason to question "viral load" data is that "viral load" **implies** the existence of viremia, i.e. the presence of virus particles in the peripheral blood, although **no one has ever observed, by** EM, **one single retroviral particle in** the blood of HIV/AIDS patients, **even in those patients tagged as presenting with a "high viral load."**

Moreover, the PCR **methods** used for "viral load" determination **bypass** the problems of **isolation** of retroviral particles. The question therefore arises: **what is actually measured in "viral load" determinations?** To date, **no** satisfactory answer has been provided. Still, various amounts of **claimed** retroviral nucleotide sequences are routinely identified and quantified in a patient's plasma. They are **interpreted** as originating from HIV, and used in the clinical assessment and therapy of AIDS patients. When Luc Montagnier was **asked**, **"What is actually measured in viral load assessments?"** during the discussion of a major HIV/AIDS debate in the European Parliament in 2003, his answer was less than - clear and convincing. The **contradiction** remains that , genomic retroviral sequences are routinely recognized by PCR, and **interpreted** as

originating from HIV particles, **while nobody has actually visualized them by EM.** More **critical** attention should be given to the **true** nature of these retroviral sequences, the **origin** of which is at present **unclear**. All the **images** of particles **supposedly** representing HIV and published in **scientific** as well as in lay publications , are derived from EM studies of cell cultures. They **never** show HIV particles coming **directly** from an AIDS patient. **The pictures are always embellished by computerized image reconstruction, with attractive colors and refined three-dimensional effects.** The endless, worldwide publication in the media of these elegant **artifacts** has done much to **persuade** scientists and lay people alike to accept the **existence** of HIV as a key part of the orthodox consensus. **Cell cultures have been the major tool that permitted the development of modern virology.** Unfortunately, these cultures are frequently **contaminated** by **microorganisms** such as mycoplasma, readily**???** identifiable by EM. These **contaminants**, well known and documented for a **long** time, frequently made the **interpretation** of experimental data rather laborious, because to demonstrate the cytopathic effects of a given virus **on cultured** cells, it would have been much preferable to experiment with "**clean**" (i.e. virus-free) cells.

The 1983 study from Institut Pasteur in Paris is illustrated by an EM showing budding retroviruses on the surface of

human cord blood lymphocytes. The **interpretation** of this figure by Luc Montagnier and his team, that these retroviruses **originated** from a pre-AIDS patient, was **based on** the fact that the cord blood lymphocytes were **exposed** to the cell-free supernatant of **"infected" co-cultures.** But the authors did **not** provide any **evidence** for "infection" in their co-cultures, **nor** for the presence of retrovirus particles **in the supernatant** of these cultures. Therefore, **another** explanation for the **origin** of the observed retroviruses on the surface of these cultured cord blood lymphocytes **must be sought.**

Drugs such as azidothymidine (AZT), a DNA chain "**terminator**," **as well as** non-nucleoside analog RT **inhibitors** (such as nevirapine) and protease **inhibitors** (such as ritonavir), are currently used in various combinations such as "highly active retroviral therapy".

(2) Observation of Retroviral Particles by Transmission Electron Microscopy

(3) The **Claimed** Efficacy of **Anti**retroviral (ARV) Drugs. Journal of American Physicians and Surgeons Volume 15 Number 3 Fall 2010 (HAART), and **repeatedly claimed to be "life saving."** Manufacturers of these drugs, however, strongly emphasize their **toxicity**.
Lethal effects of AZT became dramatically evident when mortality of seropositive hemophiliacs suddenly

increased sharply in 1987, precisely at the time high dosages of AZT started to be prescribed.

Hopes that AZT might have preventive value were **shattered** by the Concorde study, when mortality of AZT recipients was found 25% higher than that of the **untreated** control group of **symptom-free HIV-positive** individuals. These important studies have been **reviewed** by Duesberg, by Hodgkinson, and others. Equally perplexing is that deaths of ARV-treated patients very frequently result from **acute liver failure,** conflicting with the fact that HIV is **not** known for liver toxicity, whileARV drugs **are**. If the effects of ARV drugs could still be regarded as proving that HIV is the cause of AIDS, one would at least expect some patients to be cured by these drugs. However, **not a single case of "cure"** has ever been reported. Instead, the clinical evidence points to the **high toxicity** of ARV drugs &their immunodepressive effects which actually **mimic** AIDS itself. Patients with **severe** AIDS have frequently been **reported to be** transiently, but remarkably, **improved** by ARV drugs. Such "Lazarus" type observations have been **interpreted** as **evidence** for an antiretroviral **effect** on HIV, supporting the **existence**/role of HIV. However, as **most** of these patients frequently suffer from pneumonia, or mycosis, or both, and because protease inhibitors, introduced in antiretroviral therapy in 1996, have marked anticandidal **and** antipneumocystis effects??, this

interpretation is **questionable** at best. When anti-proteases help **block** ?? such opportunistic infections, this has no direct relevance to HIV, and **certainly does not** "automatically support the "HIV model." Maneuvering for major federal budget allocations, AIDS public health policies have been relying on **media amplification of fear.** Catastrophic prediction of heterosexual transmission of the disease, prophecies of a **worldwide pandemic**, and reliance on CDC and WHO statistical reports were all linked to the **assumption** that AIDS was a contagious disease, possibly transmitted in the general population by sexual intercourse.

In 1974, **Stewart** was part of a government-appointed team tasked with investigating experimental, **now-banned whooping cough vaccines given by the British government to orphans and mentally handicapped children.** In 1984, he was commissioned to write a report on the whooping cough vaccine by Britain's chief scientific officer, **but the report was never made public by** the then-British Health Secretary Kenneth Clarke.
In the 1970s, Stewart became well-known for his **opposition** to the pertussis vaccine, which he claimed to have supported **until** 1974, when he saw many vaccinated children who had **still** developed pertussis. By the mid-1970s, his criticisms of vaccines had attracted the attention of many parents who felt that their children had been injured by vaccines. With his support, the

Association of Parents of Vaccine Damaged Children was **formed** in 1973. In 1977, he published a paper citing the cases of many of these children as proof that the **DPT vaccine caused brain damage.** In 1978, he claimed at a news conference that **"As with many other infectious diseases, there was a great decline in the rate of pertussis mortality before any vaccine was available."** He also claimed that it was **safer** to get pertussis than to receive the vaccine. In 1977, Stewart criticized the British government's Committee on Safety of Medicines for calling for a pertussis vaccination campaign. He argued that **"There are no grounds for saying a major epidemic is on the way and I don't agree with the way their figures have been collected."** In the next two years, however, over 100,000 children were hospitalized with pertussis, and 600 of them died. In the mid-1980s, Stewart served as the lead witness for the prosecution in the case of Johnnie Kinnear, whose **mental** development was allegedly stunted due to his receipt of the pertussis vaccine.

1982 WRC-TV news report "Vaccine Roulette", where his work was portrayed favorably.

A former World Health Organization advisor on AIDS, Stewart was described as **one of the two** HIV/AIDS "dissidents to a degree" by the Guardian in 2000 (the **other** being Andrew Herxheimer). Stewart and Herxheimer both served on Thabo **Mbeki's** presidential advisory panel on AIDS. In a 1990 Dispatches episode

about HIV/AIDS, Stewart claimed that HIV had not spread significantly among heterosexuals in either the United States or Great Britain, **dismissing the disease's spread in Africa as "something else".**

In 2000, he told the Guardian: "We have been criminally irresponsible - we have told people they have Aids when they are HIV positive and that's **not** true. **We have told them there is no cure and no vaccine and they are going to die. We have caused endless stress and even suicide.** Families have worried about whether their children are going to be infected. That's why it is such a **panic disease. The medical establishment has made the panic."**

Stewart served as a surgeon-lieutenant in the Royal **Navy** from 1943 to 1946. He then held several hospital appointments, including senior registrar and tutor at the Wright-Fleming Institute at St Mary's Hospital, London from 1948 to1952,where he worked alongside **Alexander Fleming.** He became professor of pathology and bacteriology at the University of Karachi in 1952. He served as a consultant pathologist to the South West Metropolitan Regional Hospital Board of the National Health Service, as well as head of laboratories at the Medical Research Council Laboratories at Carshalton, from 1954 to 1963.[2] He then traveled to the United States, where he served as Professor of Epidemiology and Pathology at the University of North Carolina, Chapel Hill until 1968, and as Watkins Professor of

Epidemiology at Tulane University Medical Center until 1972. From 1972 to 1984, he was the Henry Mechan Professor of Public Health at the University of Glasgow.

- Gordon T Stewart 'paper -- Limitations of the Germ Theory -- published in Lancet May 18 1968 ... Koch's postulates of diseases were a gross simplification, as they ignored the complexity of other factors that determine if and how a disease arises.

Renowned epidemiologist **Gordon T. Stewart** did much, however, to **dispel** these erroneous predictions. In a letter, he stated "the UK Government is beginning to retreat from its pessimistic certainty about pandemics of heterosexual transmitted AIDS" and exposed to scrutiny "the claim that AIDS has **already** spread by heterosexual transmission to the general populations." Stewart's conclusions correlate well with the **complete absence** of HIV among **female** sex workers **not** using IV drugs. This "prostitute paradox" (i.e. no increased risk for AIDS among female sex workers) was reviewed from **worldwide** studies by Root-Bernstein in 1993, and re-emphasized more recently by Etienne de Harven and JeanClaude Roussez. The **lack of evidence for heterosexual transmission** of AIDS was clearly presented by Padian et al., who could not observe one single case of seroconversion in a follow-up study of 175

HIV-serodiscordant couples over a period of six years. … Safe-sex practices (e.g. condoms) remain essential, however, for the prevention of diseases proven to be sexually transmitted, such as syphilis and gonorrhea.

Certain African countries, such as Uganda and Tanzania, had been regarded as epicenters of an AIDS "pandemic." The **lack** of evidence supporting this, **initially** recognized by Philippe Krynen, was **clearly documented by** Charles Geshekter and by science writers Celia Farber and Neville Hodgkinson. The **most** authoritative conclusions presented in 2008 by experienced epidemiologist **James Chin,** former Chief of the Unit of the Global Programme on AIDS of the World Health Organization (WHO) in Geneva, in his **book** brought to a close any possible debate on heterosexual AIDS transmission. Chin stated that AIDS was, and still is **restricted** to a small population of homosexuals **and intravenous** drug users, and that the heterosexual population is **not** at risk. Chin's conclusions have **raised serious questions on the reliability of WHO statistics.** AIDS epidemiological data have been **further** confused by several consecutive **changes in the official definition of** the syndrome, and have **failed** to support the current HIV=AIDS dogma. The **hypothesis** of an exogenous retrovirus "HIV" causing AIDS appears **unsupportable** by the scientific evidence concerning molecular markers, EM findings, ARV drugs, and epidemiology.

The Collision of Epidemiology with **Political Correctness.** "Viral Loads" and Retroviral Sequences.

Journal of American Physicians and Surgeons Volume 15 Number 3 Fall 2010 .. **Since 1996, real-time PCR has been used to claim** quantification of a **postulated** HIV viremia, termed "viral load," in AIDS cases. These methods have been based on the study of patients' plasma samples: **initially**, samples originated from nuclei of peripheral blood mononuclear cells, and **later** from low-speed **centrifugation** pellets of plasma. **The various methods applied to** the PCR measurement of the **so-called "viral load"** have **one point in common: they all bypass direct isolation** of retroviral particles demonstrable by EM. These methods are **not** expected to **isolate**, **nor** concentrate any retrovirus. Moreover, **as clearly stated** during the South African 2000 conference, **not one** single particle of retrovirus has **ever been seen,** by EM, in the blood plasma of **any** AIDS patient, **even in those patients identified as presenting with a high so-called "viral load."** That statement, widely publicized, has **never** been **refuted nor challenged.** Human plasma carries various amounts of circulating DNA. Suspected for a long time, this was **first** demonstrated by modern technologies in 1999, by P. Anker et al., in the blood of **cancer** patients.

The significance of circulating nucleic acids, as possible molecular markers in the study of cancer, was **extensively reviewed** in a NewYork Academy of Sciences conference in 2006. The **origin** of free circulating DNA is **complex**, and **seems** to depend **primarily** on "cell" apoptosis. "If the engulfment of apoptotic bodies is **impaired** or cell **death** is increased enough , to produce substantial amounts of circulating DNA, **inflammation** would definitely be a problem and autoimmunity would occur frequently in cancer and **other** conditions involving increased circulating DNA." Apoptosis **and a large** spectrum of infectious diseases are constant components of all clinical AIDS cases. **Circulating** DNA is expected, therefore, in the plasma of all symptomatic AIDS patients. Amounts can vary, as a function of more or less **rapid removal** of DNA by clearance mechanisms. Apoptotic bodies and/or fragments of PBMC nuclei are certainly expected in **low-speed centrifugation** plasma pellets, such as those used in PCR "viral load" measurements, and most likely **increase** the amount of recognizable DNA. Human DNA always contains approximately 8% of retroviral nucleotide sequences. It's no surprise, therefore, that RT-PCR study of plasma pellets **shows, and amplifies,** retroviral nucleotide sequences. Unfortunately, such findings are frequently **misinterpreted** as originating from hypothetical exogenous "HIV," although, as stated above, **not one single** retroviral particle has ever been found by EM in plasma samples.

Quantifying a **presumed** "viral load" has, therefore, probably **nothing to do with an exogenous** "HIV." **It simply reflects variable amounts of circulating** DNA. Retroviral sequences in plasma pellets being **easily** explained by the presence of variable amounts of circulating DNA, one should **not**, however, expect that these nucleotide sequences would be **identical** in all cases. Quite to the contrary, since "nucleotide sequences that diverged from co-linearity with the typical retroviral genome (LTRgag-pol-env-LTR) considerably increase the number of HERV families," the large number of HERV families resulting apparently from frequent recombinational deletions. Expected variations in the observed nucleotide sequences have, unfortunately, often been **misinterpreted** as an indication for a high rate of HIV mutations! It seems much more likely, however, that the numerous variations in the observed retroviral nucleotide sequences in circulating DNA **reflect** the large number of HERV families they originate from, and have **nothing** to do with **presumed** "mutations" of a **hypothetical** HIV.

Reference to HERVs and/or to circulating DNA can **hardly be found in the extensive literature on "viral load" measurements,** interference of HERVs, and of circulating DNA being consistently **ignored** by the HIV/AIDS **orthodoxy**.

Conclusively, RT-PCR identification, and **presumed** quantification of so-called "HIV viral load," can easily be explained by the variable amounts of HERV-derived retroviral nucleotide sequences present in the circulating DNAofAIDS patients. In their 1983 paper, **Barré-Sinoussi** et al. **failed** to demonstrate, by EM, any retrovirus in their co-cultures. Still, the supernatant of these co-cultures has been **used** to **"infect"** human cord blood lymphocytes. **This theory requires one to subscribe to infection via a virus that is not visible by EM.** If the authors had included EM evidence for retroviruses in their co-cultures and their supernatant, their interpretation would have been more convincing. Unfortunately, such data were **not** provided. Nevertheless, their Figure 2 unquestionably demonstrates "budding" retroviruses on the surface of cultured human cord blood lymphocytes. Its **origin** needs to be better clarified. Cord blood lymphocytes are placenta-derived cells. **The human placenta is well known for its high content of HERVs,** with EM recognizable retrovirus particles. Cord blood lymphocytes are, therefore, likely to carry **similar** HERVs. The 1983 paper demonstrated that HERV particle expression had been successfully activated in cultured cord blood lymphocytes, under culture conditions that included 2g/ml of Polybrene. It does **not** demonstrate, however, that the EM-observed retroviruses originated from the studied pre-AIDS patient. A **long-overdue control experiment** would be to study, by EM,

cultured cord blood lymphocytes **under conditions that would reproduce exactly** those used at the Pasteur Institute in 1983. **Dourmashkin** presented some data addressing this issue in 1992, although his presentation did not satisfactorily resolve the problem, since his cord blood lymphocytes were **not** cultured under conditions identical to those used at Pasteur in 1983. The EM observation of typical retroviral particles in the 1983 Pasteur paper can **alternatively** be explained by the presence of placenta-derived, Polybrene-activated HERVs. However, this EM observation does **not** support the existence of an AIDS-related, exogenous retrovirus. Obviously, **confounding** by HERVs **cannot** be ignored in the objective analysis of clinical as well as basic HIV/AIDS research. All AIDS Rethinkers are united in the fundamental opinion that HIV **is not the cause of AIDS.** However, they **diverge** on the **important** question of the very **existence** of the Human Immunodeficiency Virus (HIV). Some of them maintain that HIV is a "harmless passenger virus," while others claim that HIV "does not exist" at all. Since **neither** of these two positions explains the **pertinent** observations, an **alternative interpretation, compatible with all the available scientific evidence, is needed.**

Retroviruses on the Surface of Cord Blood Lymphocytes Discussion Science . Journal of American Physicians and Surgeons Volume 15 Number 3 Fall 2010.

Claiming that HIV is a harmless passenger virus raises at least two critical problems. First, if HIV is "harmless" it cannot be linked to immune deficiency (a very severe pathological condition), as implied in its name. Therefore, the name of the virus should at least be changed in order to fit with a claimed "harmless" character. Secondly, in the general classification of **animal** virology, very large numbers of viruses are **non**pathogenic, as was well illustrated in the 1960s in a special conference, at the NewYork Academy of Sciences, under the title **"Viruses in Search of Diseases."** Obviously, all nonpathogenic (i.e. "harmless") viruses are clearly **visible** under the EM**??. Pathogenic and nonpathogenic viruses look identical under the EM.** In AIDS research, retroviral particles**??** were observed by EM **only in complex cell culture systems, never directly in the plasma, nor in the tissues of any AIDS patient.**

HIV should **not** be considered an HERV, since the **hypothetical** HIV is supposed to be an exogenous, infectious microorganism, while HERVs are fundamentally **endogenous**, **non**-infectious, vertically transmitted, **defective** "viruses". Still, HERVs have been a "confounding" factor in HIV/AIDS research, and have caused **confusion** in **interpreting** the **concept of "viral load."** Moreover, HERVs put HIV researchers on the **wrong** track, creating the **illusion** of continuous HIV

mutations—mutations that **improperly** served to explain the extreme difficulty in preparing anti-HIV vaccines. However, difficulties in developing anti-HIV vaccines might not be explained by a constantly mutating HIV, but rather by a lack of exogenous HIV.

As emphasized years ago by Papadopulos, Lanka, and others, there is no scientifically verifiable evidence to confirm the existence of a hypothetical exogenous HIV.

Sloan Kettering = Rockefeller.

Etienne de Harven, M.D., , Brussels University (ULB), 1953 became a full member of **Sloan Kettering** Institute, New York, N.Y., in 1968, and is emeritus professor of pathology, University of Toronto, Toronto, Ontario. Contact: pitou.deharven@orange.fr Conflicts of interest: Acknowledgment. REFERENCES **none**. The author wishes to express his gratitude to Prof. Gordon T. Stewart (Edinburgh) and to Dr. Christian Fiala (Vienna) for their critical review of the manuscript, and for many constructive discussions on AIDS over the past 15 years. **Gallo** R, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk of AIDS. **Montagnier** L. Lymphadenopathy-associated virus: from molecular biology to pathogenicity. Essex M, McLane

MF, Lee TH, et al. Antibodies to cell membrane antigens associated with human T-cell leukemia virus in patients with AIDS. Duesberg PH. Retroviruses as carcinogens and pathogens: expectations and reality. Duesberg PH. Washington, D.C.: Regnery; 1996. Sonnabend JA. Caution on AIDS viruses. 1984. Stewart GT. Uncertainties about AIDS and HIV. 1989. **Mullis K.** Foreword. In: Duesberg PH. Washington, D.C.: Regnery; 1996. Lang S. New York, N.Y.: Springer Verlag; 1997. Papadopulos-Eleopulos E. Reappraisal of AIDS—is the oxidation induced by the risk factors the primary cause? 1988;25:151-162. Rasnick D. Inhibitors of HIV proteases useless against AIDS—because HIV doesn't cause AIDS. , August 1996. Available at: www.virusmyth.com/aids/hiv/drinhibit.htm . Accessed Apr 7, 2020. Geshekter CL. Reappraising AIDS in Africa —underdevelopment & racial stereotypes. , September/October 1997:5. Available at: www.virusmyth.com/aids/hiv/cgreappraising.htm . Accessed Apr 7, 2020. Farber C. Sins of omission—the AZT scandal. , November 1989. Lauritsen J. AZT on trial. , Oct 19, 1987. Hodgkinson N. African AIDS: true or false? (London), Sep 5, 1993. Shenton J. London-New York: IB Tauris; 1998. Maggiore C. 4th ed. American Foundation for AIDS Alternatives; 2000. Russeil R. [in French]. ChêneBourg/Genève: Editions Vivez Soleil; 1996. Tahi D. Interview with Luc Montagnier—**Did Luc Montagnier discover HIV?** , Winter 1997. Available at:

www.virusmyth.com/aids/hiv/dtinterviewlm.htm . Accessed Apr 7, 2020. Roussez JC. [in French]. 2nd ed. Belgium: Marco Pietteur; 2005. Roberts J. Bristol, UK: Impact Investigative Media Productions; 2009. Christie H. **Do antibody tests prove HIV infection?** , winter 1997. Available at: www.virusmyth.com/aids/hiv/hcinterviewvt.htm . Accessed Apr 7, 2020. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. **Is a positive western blot proof of HIV Infection?** 1996. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. **Has Gallo proven the role of HIV in AIDS?** 1993. Papadopulos-Eleopulos E, Turner VF, Papdimitriou JM, Causer D, Page B. HIV antibody tests and viral load—more unanswered questions and a further plea for clarification. 1998; Mbeki T. Presidential AIDS Advisory Panel Report. A synthesis report of the deliberations by the panel of experts invited by the President of the Republic of South Africa, the Honourable Mr. Thabo Mbeki, March 2001. Lannoye P. [in French]. Proceedings of the Colloquium held at the European Parliament, Brussels, Belgium, Dec 8 2003. Embourg, Belgium: Marco Pietteur; 2004. **Fauci** AS, Johnston MI, Diefenbach CW, et al. HIV vaccine research: the way forward. Chigwedere P, Essex M. Aids denialism and public health practice. 2010

http://www.theperthgroup.com/links.html
https://www.virusmyth.com/aids/hiv/panel/aidsreport.pdf
www.robertogiraldo.com/eng/Websites.html

Science Ann Intern Med Science Cancer Res **Inventing the AIDS Virus.** Nature Lancet Inventing the AIDS Virus. Challenges. Med Hypothesis Reappraising AIDS Reappraising AIDS Spin New York Native Sunday Times Positively False—Exposing the Myths around HIV and AIDS. **What If Everything You Thought You Knew about AIDS Was Wrong?**

The chemical bases of the various AIDS epidemics: recreational drugs, anti-viral chemotherapy and malnutrition. 2003; The Durban Declaration. 2000; Baltimore D. Viral RNA-dependent DNA polymerase in virions of RNA tumor viruses. 1970; Temin HM, Mizutani S. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. 1970; Varmus H. Reverse transcription. 1987; Bess JW, Gorelick RJ, Bosche WJ, Henderson LE, Arthur LO. Microvesicles are a source of **contaminating** proteins found in purified HIV-1 preparations. 1997; Gluschankof P, Mondor I, Gelderblom HR, Sattentau QJ. Cell membrane vesicles are a major contaminant of gradient-enriched human immunodeficiency virus type-1 preparations. 1997; Abbott Laboratories. Human Immunodeficiency Virus Type 1. Abbott Laboratories, Diagnostic Division 66-8805/R5, January 1997:5. Hodgkinson N. New doubts

over AIDS infection as HIV test declared invalid. (London), Aug 1, 1993. Johnson C. **Whose antibodies are they anyway?** 1996:4(3):4. Available at: **www.altheal.org/continuum/Vol4no3.pdf** . Accessed Apr 7, 2020. Hodgkinson N. **HIV diagnosis: a ludicrous case of circular reasoning. ,** May 21, 2006. Giraldo R. **Everybody reacts positive on the ELISA test for HIV. ,** 1999;5(5):9-11. Available at: www.altheal.org/continuum/Vol5no5.pdf . Accessed Apr 7, 2020. Tahi D. Interview Luc Montagnier-Did Luc Montagnier discover HIV? , 1997;5(2):30-39. Available at: www.virusmyth.com/aids/hiv/dtinterviewlm.htm . Accessed Apr 7, 2020. Mortimer PP. **The fallibility of HIV western blot.** 1991;337:286-287. De Harven E. **Problems with Isolating HIV.** European Parliament, Brussels, Dec 8, 2003. Available at: www.altheal.org/isolation/isolhiv.htm . Accessed Apr 7, 2020. Bauer H. **HIV Tests are not HIV tests.** 2010;. De Harven E. Summary statement. Interim Report of the AIDS Advisory Panel, Pretoria, South Africa, May 2000. Complete report available at: www.altheal.org/africa/aidspanelreport.pdf . Accessed Apr 7, 2020. Gelderblom HR. Assembly and morphology of HIV: potential effect of structure on viral function. 1991; Fogh J. . London-New York: Academic Press; 1973. Barré-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency (AIDS). 1983;

Darby SC, Ewart DW, Giangrande PL, et al. Mortality before and after HIV infection in the complete population of haemophiliacs. UK Haemophilia Centre Directors' Organisation. 1995; Philpott P. Darby debunked—**pro-HIV hemophiliac study actually points toward non-contagious AIDS.** S 1996;:4(Feb):1-4. Available at: http://voluntarysociety.org/action/other/hr/ra/1996-02%28Feb%29DarbyHemo.pdf . Accessed Apr 7, 2020. Seligman M, Chess L, Fahey JL, et al. (Concorde Coordinating Committee). Concorde: MRC/ANRS randomized double-blond controlled trial of immediate and deferred **zidovudine** in symptom-**free** HIV infection. 1994; Hodgkinson N. London, England: Fourth Estate Limited;1996. Justice AC, Wagner JH, Fusco GP, et al. HIV survival: liver function tests independently predict survival. 14th International AIDS Conference, Barcelona, Spain, July 2002. Cassone A, De Bernardis F, Torosantucci A, et al. In vitro and in vivo anticandidal activity of human immunodeficiency virus protease inhibitors. 1999; Atzori C, Angeli E, Mainini A, et al. In vitro activity of human immunodeficiency virus protease inhibitors against . 2000;. Philpott P. Might HAART help? Benefits from blocking opportunistic infections, not HIV. 2000;8(9):1-2. Nature Int J STD AIDS J Biosci Nature Nature Nature CR Acad Sci Sci Am Virology Virology Sunday Times Continuum The Business Continuum Continuum Lancet J Am Phys Surg AIDS **Contamination** in Tissue Culture Science Nature

Rethinking AID Lancet AIDS–The Failure of Contemporary Science: **How a Virus That Never Was Deceived the World.**
Stewart GT. Errors in predictions of the incidence and distribution of AIDS. 1993; Stewart GT. The epidemiology and transmission of AIDS: a **hypothesis** linking behavioural and biological determinants to time, person and place. 1995:. Root-Bernstein R. The **prostitute** paradox. , March, 1993. Available at: www.virusmyth.com/aids/hiv/rrbprostitute.htm . Accessed Apr 7, 2020. De Harven E, Roussez JC. Trafford Publishing; 2008. Padian NS, Shiboski SO, Glass SO, Vittingoff E. Heterosexual transmission of human immunodeficiency virus (HIV) in Northern California: results from a ten-year study. 1997:. Fiala C. [in German]. Vienna, Austria: Deuticke Verlag; 1997. Hodgkinson N. **The plague that never was.** (London), Oct 3, 1993. Geshekter CL. **Myths and misconceptions of the orthodox view of AIDS in Africa.** 2007;. Farber C. Out of Africa. , March 1993. Fiala. C. AIDS in Africa: a call for sense **not hysteria.** Presentation at RA2009, Oakland, Calif., November 2009.

Chin J. Oxford, UK: Radcliffe Publishing; 2007. Nelson PN, Carnegie PR, Martin J, et al. **Demystified. Human endogenous retroviruses.** 2003:. Leib-Mosch C, Brack-Werner R, Werner T, et al. Endogenous retroviral elements in human DNA. 1990; Medstrand P, Lindeskog

M, Blomberg J. Expression of human endogenous retroviral sequences in peripheral blood mononuclear cells of healthy individuals. 1992;. Lower R, Lower J, Kurth R. Review.
Caliendo AM. Methods, **interpretation** and applications of HIV-1 viral load measurements. 1997;. Russell A. The Hue Christie Memorial Prize: $100,000 reward for "HIV." Available at: www.altheal.org/isolation/prize.htm . Accessed Apr 7, 2020. Anker P, Mulcahy H, Chen XQ, Stroun M. Detection of circulating DNA in the blood (plasma/serum) of cancer patients. 1999;18:65-73. Swaminathan R, Butt A, Gahan P. Circulating nucleic acids in plasma and serum IV 2006;1075:1–353. Van Der Vaart M, Pretorius PJ. The **origin** of circulating free DNA. 2007;. Belshaw R, Watson J, Katzourakis A, et al. The rate of recombinational deletion among human endogenous retroviruses (HERVs). 2007;. De Harven E, Friend C. Further electron microscope studies of a mouse leukemia induced by cell-free filtrates. 1960;. Dourmashkin R, Bucher D, Oxford JS. Small virus-**like** particles bud from the cell membranes of normal as well as HIV-infected human lymphoid cells. 1993;. Stewart GT, Mhlongo S, de Harven E, et al. The Durban declaration **is not accepted by all.** 2000;. Voisset C, Weiss RA, Griffiths DJ. Human RNA "**rumor**" viruses: the search for **novel** human retroviruses in chronic disease 2008;. Lanka S. **HIV: reality or artefact?** April/May

1995. Available at: **www.virusmyth.com/aids/hiv/slartefact.htm** . Accessed Apr 7, 2020. De Harven E. Viral etiology of human cancer: a historical perspective. 1998;. **Spin, Modern Medicine—The New World Religion: How Beliefs Secretly Influence Medical Dogma and Practices** Lancet Genetica Rethinking AIDS **Ten Lies About AIDS.** Am J Epidemiol Lieben wir gefährlich? [Do We Love Dangerously] Sunday Times Ethics & Politics Spin The AIDS **Pandemic—The Collision of Epidemiology with Political Correctness.** Mol Pathol Cancer Res J Gen Virol Proc Natl Acad Sci USA Reprod Sci Curr Top Pathol J Virol Clin Microbiol Rev Nucleic Acids Res Clin Microbiol Newsletter Cancer Metastasis Rev . Ann NY Acad Sci Clin Chem J Virol J Biophys Biochem Cytol J Med Virol Nature . Microbiol Mol Biol Rev Continuum, Haematologica 74 Journal of American Physicians and Surgeons Volume 15 Number 3 Fall 2010 https://www.jpands.org/vol15no3/deharven.pdf

CAN ELECTRON MICROSCOPY RESOLVE THE HIV BATTLE? AN EXCLUSIVE INTERVIEW WITH EM PIONEER DR. ÉTIENNE DE HARVEN
ARCHIVE INVESTIGATIONS JUNE 20, 2013

For scientists like Dr. de Harven, "what Gallo did," and all that followed, represents a kind of holocaust on

all they'd known and taken for granted: Empirical, classical science.

Now retired, Dr. de Harven's **life's work has revolved around Electron Microscopy,** a pioneering technology now at the center of the explosive trial in Buffalo New York, where **Nushawn Williams,** after serving 12 years in prison, and still being in state custody due to "mental abnormalities," he is said to possess, has been found to be HIV free on electron microscopy (EM) tests.

Amidst political machinations surrounding the attempted media blackout from the trial– coercion and even possible witness tampering; the internet is ablaze with new disputes as to whether being HIV negative on EM is a "valid" way to be considered HIV free, or negative. As we all know, **the church is built entirely upon the now discredited HIV antibody tests and PCR viral load tests, both of which state as disclaimers in package inserts that they are not designed to test for HIV.**

The Truth Barrier contacted Dr. de Harven as well as The Perth Group, with questions about the Nushawn Williams case and how it relates to the HIV existential debate, and these materials will be published as the story unfolds.

There is disagreement about how EM tests must be performed in order to properly discern whether HIV is present or absent in the blood.

But what does this thing, not word, bit of code–"HIV"–mean?

The "debate" about HIV's **causation** has been, I'm devastated to realize, obscured by the sheer fact that we used as concepts, symbols, words that **lacked** meaning. We began by saying "Does HIV cause AIDS," ***when we should have said,* "What do we mean when we say "HIV*?"***

If you have the mental stamina to unravel it, please read the writings collected at The Perth Group's website. http://www.theperthgroup.com/ I have certainly failed on this front–trying to unravel *this*. It was the hardest part of the gigantic knot, but it was the most important, by far. The knot ties all perceptions, illusions, shadows, linguistics, hallucinations, and stunning truths together at its center: HIV's existence (as exogenous "retrovirus," in human blood, **not as technological artifact**,) the "validity" of the "HIV test," the question of "cause" and pathogenicity, and the question of infectivity.

To accusations that I have not dealt with this adequately, I am guilty as charged.

In other words: I have mostly (not entirely) wasted the past 27 years of my life as an AIDS unraveler, **because I did not start at the epicenter:** The existential question– **Not *does* it, but *is* it?**

"Does HIV Cause AIDS?" **already contains accumulated detritus, constructed words, and what William Burroughs called, the "set up."**

The Truth Barrier is delighted to bring you this Q&A with a **true expert** on EM. Dr. de Harven is the former President of the Electron Microscopy Society of America. [Note to readers: 1. The questions were emailed and responded to in writing, not asked in sequence, so there will be passages where the question asked suggests that the interviewer has not heard or comprehended the answers given.]

INTERVIEW

Q: Tell us about electron microscopy, EM: What is it? What need did it answer when it was pioneered, and what role did you play in it?

A. There are several types of electron microscopes (EMs). One that brought, by far, the most important contributions to bio-medical research is the "transmission" electron microscope (TEM).

I shall, therefore limit my remarks to the **birth** of TEM. The inventor of TEM is **Ernst Ruska**, who constructed and successfully operated the first TEM, in collaboration with **Knoll**, in Berlin at the research laboratories of the **Siemens** CY, in 1931. The instrument was aimed at developing a microscope offering a "resolution" better than that of the optical microscope. **By** "resolution", we mean the shortest distance separating two punctual objects that could still clearly be recognized as two, and not one. The resolution of the optical microscope is

limited at the level of 0.2 micrometer ("microns"; one micron = one thousandth of a millimeter). This limitation was recognized as making the optical microscope totally unable to visualize **viruses**. By contrast, the resolution of the TEM is around one Angström unit. One micrometer equals 10.000 Angström. It follows that the resolution of the TEM is approximately one thousand times better than that of the optical microscope, making the **direct??** visualization of viruses, and even of single **atoms??** possible. **Most viruses are definitely smaller than 0.2 micrometer.???** The size (diameter) of viruses was **approximated??** before they were actually visualized, from the analysis of the average pore size of ultra filters through which these viral particles could go through, testing the infectivity of "ultra-filtrates."

Ruska definitely demonstrated, in 1931-32, that his electron microscope had, indeed, a resolution better than that of the optical microscope. His microscope (the first TEM) was using a beam of **electrons???** instead of a beam of light, and using electro-magnetic fields, **generated by several magnetic "lenses", to deflect there pathway instead of optical lenses.???** The technology involved in this instrument has **several** points of similitude with that of the cathodic TV tube. For this discovery, Ruska received the Nobel Prize in **1986**, two years before he died.

The hope of Ernst Ruska was that his new microscope could, possibly, visualize viruses, and he actually was thinking about the poliovirus! ??????????

Still, in the mid **1930s**, many biologists thought that this new microscope could **never** be useful in biological research, fearing that **biological specimens would inevitably be destroyed by the electron beam–like burned out by a lightning.**

This was demonstrated to be **false ????** at the University of Brussels, Belgium, in 1936, by **Louis Marton who published the first EM images of cells, taken with an EM of "his own" making.**

The **first** EM made in America was constructed at the University of Toronto, Toronto, Ontario, at the Banting Institute, in **1938**. Important improvements, aimed at **correcting** some image defects, were developed within the laboratories of the RCA CY, in Camden, PA, during **WWII**.
Just after WWII, **Albert Claude** (Nobel, 1976), using an RCA microscope and working at the **Rockefeller** Institute in New York, succeeded in imaging the **Rous sarcoma virus,** observed **within** infected, cultured cells. This was **definitely** the **first** application of EM to the direct visualization of **what we now call retroviruses**. In 1955, I was fortunate to operate the very **first** "Elmiskop

I" from the **Siemens** CY in the USA, installed at the **Sloan Kettering** Institute, in New York City, and with which I made **early** contributions (1956-1960) to the ultrastructure of murine leukemia **viruses**, to their "budding" phenomenon, and to their purification from the blood plasma of leukemic mice. That microscope had a resolution far **superior** that of the US made **RCA Radio Corporation of America** instruments.

By the early 1960s, **all the known viruses** had been well characterized under EM, and, **unquestionably**, TEM has been a **major** factor in the **emergence** of **modern virology**, as well as of **modern cell** biology.
Nobelist André Lwoff recommended, at a Cold Spring Harbor conference in 1962, that the general classification of all **viruses** be primarily **based** on their morphology, as seen by EM.

By **1970** however, and **in spite** of a most extensive, worldwide research effort, **not** a single virus was ever demonstrated by TEM to be significantly associated with any form of human cancer or leukemia.

Q: What happened exactly vis a vis "HIV" and EM, in 1983/84? Was patient blood serum (HIV Elisa/WB positive) validated against EM? Ever? It seems to me they say that yes, it was, yet we have **Montagnier saying,**

“We did not purify.” Let me elaborate the question a little:
What should have happened, vs. what did happen, with the **“new virus” and** EM, in this period of time?

A. What happened in **1983** is a **direct** consequence of what developed since 1970, i.e. **the highly predominant reliance on “molecular markers”.**
Unacceptably frustrated by the **total lack of success** in all attempts to demonstrate virus particles in human cancer by EM, the “impresarios” of the cancer/virus “dream” (**Gallo, Fauci, and** others) totally engaged in the molecular approach.
Consequently, they **invented** molecular markers to compensate for the **missing** viral particles… This would have been acceptable **if** the **specificity** of these **new** molecular markers would have been clearly established.
Unfortunately, this was **not** the case. The **most misleading** molecular marker was probably the **first** one, i.e. **the enzyme reverse transcriptase** (RT). Following Temin and Baltimore 1970 papers in “Science”, the RT enzymatic activity has been, **most abusively**, **used as a specific retroviral marker.** Both Temin and Baltimore demonstrated RT activity in samples of **supposedly** “purified” retrovirus.
Embarrassingly, they both **omitted** to verify the “purity” of their samples by EM. Some of their samples were simply purchased from a commercial company… True,

the label on the vials read “pure retrovirus”… However, it was known that these commercial “pure retrovirus” were **heavily** contaminated by cellular debris!
And since it is **also** known that **all** cells contain RT (see Varmus), cellular **debris** are most likely carrying similar RT enzymes.
Temin and Baltimore **did not,** therefore, prove that RT is a specific molecular marker for retroviruses. **It would have been so simple to check,???** by EM, the degree of “purity” of the samples they used. This would have, most probably, shown important cell **debris** contamination, and would have obliged Temin and Baltimore to be much more cautious in the **interpretation** of their results. In 1975, the members of the Nobel Committee, most regrettably, **failed??** to scrutinize this “purity” problem…

In 1983, at **Pasteur** Institute in Paris, **reliance on the RT marker was a key element in the claimed “isolation” of a new retrovirus.** Still, Montagnier himself recognized **“We did not purify”**… He dangerously **omitted** to consider the misleading **interference** of cell debris, **just as** Temin and Baltimore did in 1970.
But a paper on the discovery of a new retrovirus looks much better if it contains at least… one EM picture! So, members of Montagnier’s team spent **hours** at the TEM, looking at their mixed cell cultures, and they **found** the **virus**!
See Fig. 2 in their “historic” 1983 “Science” paper! It is,

by the way, a good quality EM picture. It shows **unquestionable** retroviral particles, budding at the surface of a cell. But the **legend** of this Fig. 2 states that this cell is a cord blood lymphocyte. Indeed, cord blood lymphocytes were **admixed** to these complex cell cultures (why?)

Montagnier and his co-workers **should have** known that human embryonic tissues, and the placenta in particular, are very rich in endogenous retroviruses (HERVs)**????**, and that cord blood lymphocytes should therefore be **expected** to carry the same endogenous retroviruses (under the TEM, endogenous **and** exogenous viruses, looking **identical**, **cannot** be distinguished.)

The budding of these particles has perhaps been **stimulated** by some of the growth factors also present in these cell cultures. An essential control would have been to repeat the experiment using lymphocytes from the peripheral blood **instead** of from cord blood. This control is unfortunately **missing**.

In short, I would frankly state that the Pasteur **1983** paper (whose 30th anniversary has just been celebrated in a "grand messe" of official HIV retro-virology!) **contributed very little in AIDS research because its conclusion (i.e. "the isolation of a new retrovirus") is based on ..1)the use of a non specific** RT molecular marker, and 2) is **falsely supported by** EM pictures of, **most** probably, endogenous human retroviruses.

More details and appropriate references on this analysis can be found in my 2010 paper published in the Journal of American Physicians and Surgeons (www.jpands.org/vol15no3/deharven.pdf).

Q: When antibody and VL tests became widespread as diagnostic tools for "HIV infection" over the ensuing decades, what happened with EM inside of HIV science and literature? It is my understanding that **nobody has ever found HIV in human blood, on EM.** Is this an accurate way to say it?

A: In my views, Western Blot tests lost all credibility after the publication of Eleni Papadopulos's et al. (1993) paper, and antibody tests ("Elisa") after Christine Johnson's report(1996). The **notion** of a **"Viral load"(VL),** however, brought a new parameter in AIDS diagnosis (Ho,1996). It called attention to the **actual** number of HIV particles supposedly present in the blood plasma of AIDS patients, PCR technologies being presumed to offer a way to **quantify** that number.
If such a viremia (i.e. presence of virus particles in the blood) is indeed present in AIDS patients, it reminisces the retroviral viremia well known in leukemic mice. In such case, retroviral particles should be readily demonstrable, by TEM, of **appropriately** prepared patient plasma samples. Unfortunately, **it has never been possible to demonstrate by TEM one single retroviral**

particle in the blood plasma of any AIDS patient, even if one selects patients presenting with a so-called "high viral load."

I was apparently the first researcher to make that statement, during the opening session of President T. Mbeki's major AIDS conference, in Pretoria, SA, in May 2000. My statement to that effect **has never been refuted.**

How come?

That question must be answered because "**something**" is measured by PCR technologies in the blood of many AIDS patients. Actually, **what is being measured is definitely not the number of retroviral particles (phantom-like, i.e. EM invisible!).**

In fact, what is being PCR identified, amplified, and supposedly quantified is the number of genomic nucleotide sequences that are extremely similar to sequences known to be part of the retroviral genome. Most regrettably, these sequences were **misinterpreted** as an indication as a certain number of … HIV particles! This did a lot to consolidate the **quasi-religious dogma of HIV as the cause of AIDS,** a dogma that as been sharply criticized, a few years ago, by **David Rasnick** who wrote, authoritatively, about "The AIDS Blunder"…

This **interpretation** would have been acceptable **only if** retroviral particles would have been readily demonstrated, by EM, in the blood plasma of these patients; but, since

this is **not** the case, another explanation for the presence of these nucleotide sequences has to be founded.
I presented at the RA conference in Oakland, CA, in 2009, and further developed in my 2010 JAPS paper such a much needed explanation for the presence of these retroviral-like nucleotide sequences. My explanation is **based on the well known, variable amounts of circulating** DNA in the blood of severely ill patients, and on the fact that we all carry retroviral-like sequences in our DNA, **as** endogenous, defective retroviruses, i.e. HERVs (HERVs, for "Human endogenous retroviruses") (See "Virus in all of us", R. Lower at al., 1996 PNAS paper).
No surprise, therefore, that these nucleotide sequences are recognized by PCR in the blood of many AIDS patients, who are indeed severely ill. As already demonstrated in 2008 in **Robin Weiss lab**oratory, HERVs can interfere as confounding factors in the search for novel retrovirus in chronic human diseases.
In addition, **"viral load" clinical data will remain very hard to interpret,** as long as the essential control is **missing**. The essential control would be to search for "viral load" in serologically negative (HIV-), severely ill patients, ill **from** advanced cancer or from infectious diseases, NOT **from** AIDS. Unfortunately, this essential control has **never** been done, **since** the **so-called "Viral load" has, so far, been exclusively** searched for in HIV+ patients…

Conclusively, **measuring "viral load" does not prove the existence of a hypothetical HIV.**

Q: If so, if that is true, then what does it mean? Paint a picture for us. The story of the virus, the **"new deadly virus,"** what happens first: What steps did they **Montagnier**, on one hand, **Gallo** on the other take to "find" the new entity? Then once they 'found' it, what shape was it in? It was not an entity, a thing, with a body, right? It was not coherent. Can we say that? So it **lived** where? **It was seen only through the technologies developed to find it, Elisa, WB? Later PCR/VL? But what happened back THEN when they tried to see it on EM? Why didn't** everybody look for it on EM? Too expensive?

A: No, EM is not cheap but not that expensive! And its cost has certainly nothing to do with the fact that it has **barely** been used for the past 30 years in AIDS research! It has **not** been used **because** "They" **knew it was not going to show anything of retroviral significance in samples coming directly from** AIDS patients. And since AIDS had become big business, the stocks of involved giant pharmaceutical companies could not be jeopardized! It had to be saved at all cost, even at the cost of **trusting non specific molecular markers… Fear is good business, and viruses generate fear most efficiently**… So, the HIV flag has to be maximally agitated. In

worldwide medias, with **thousands of computer-generated, colorful caricatures of an idealistic retrovirus…** By contrast, the medias have been dominated by the most rigorous **censorship** when it comes to inform the public about views of rethinking dissidents. This total **censorship** put a safety lock on any information that could jeopardize the colossal, entirely HIV derived profits of the major pharmaceutical companies.
But I am glad we have Internet!
Daring to say that HIV does not exist amounts to some sort of a capitalistic crime…
Yes, the HIV dogma is probably the **darkest** page in the history of modern medicine.

Q: What was your reaction when you saw the recent story that Nushawn Williams does not "have HIV," despite being repeatedly positive on antibody and VL tests over the decades.

A: I am not, unfortunately, familiar enough with phylogenetic analysis methodology to provide a critical view on this case. I was impressed, however, by the recent (June 1st and 2nd, 2013) e-mailings between Georg von Wintzingerode and David Crowe on this topic, and I would suggest to read that correspondence for elaborate questions on the analysis of Nushawn Williams story. Still, one point I wish to make is that, as far as I know (?),

DNA/RNA phylogenetic analysis is currently performed **exclusively** on HIV antibody positive people(?). **Where are the essential phylogenetic controls on antibody negative people?** If indeed that **control is also missing,** then the significance of such studies is **wide open for questions,** just as much as the significance of **the alleged HIV "viral load"** (see above) that has also **never** been searched for among HIV antibody **negative** patients.

Q: Etienne, if you could sum up: Does HIV exist? If so, where and how and as what?
If you could examine 1,000 HIV positive people's blood under EM, what would you expect to find? If you don't find HIV on EM in human blood, can any argument be made that the virus is "hiding" and so forth, or that the **drugs suppressed the virus** to undetectable levels? This is what the defenders of the **orthodoxy** seem to be saying about the results seen in the Nushawn Williams case.

A: This is the main question! Questioning the very existence of HIV is not something that should be debated only between specialized retro-virologists. It is an **essential** question that concerns **all** of us.
Why?
Simply because 100% of AIDS research funding is based on the **dogmatically postulated** existence of HIV. If HIV does **not** exist, it would follow that AIDS research is the most appalling case of total misappropriation of public

research funds! And it would also follow that the monumental amounts of money, so far exclusively devoted to HIV research, would be much better used in other directions. Could you imagine what world we would live in, today, if the total amount of money wasted over the past 30 years on HIV research had been, instead, used for feeding starving Africans, for clean water supply equipment, for public hygiene infrastructures, and for public health education? This would happen only if HIV research is **totally stopped!** And for this, the scientific and public health organizations **have to face the fact that, indeed, HIV does not exist!**

If, to the contrary, one keeps talking about HIV as… possibly hiding, or possibly harmless, or possibly endogenous, then the waste of research funding on HIV research shall endlessly be tolerated.

Instead, we all have to, courageously, face the fact that the **very existence of an exogenous HIV has never been scientifically verified.**

True: **to prove that something does not exist is never an easy task.** But it is the responsibility of the scientific, **orthodox** establishment to bring us the **proof of the opposite, i.e. the proof of the existence of HIV** as an **exogenous** retrovirus responsible for the **causation** of AIDS. And such a proof has to be **based on classic virology methods, not on the use of questionable molecular markers.** So far, the **hypothetical HIV has never been properly isolated, nor properly**

concentrated, nor even ever purified. As long as this remains the case, the **specificity** of HIV molecular markers shall **not** be accepted.

The Perth Group (PG, **Eleni Papadopulos and Valendar Turner et al**.) had repeatedly, in the early 1990, stressed the notion that in view of the considerable difficulty encountered in all attempts to **isolate/purify** HIV, the **specificity** of HIV molecular markers was **most uncertain.**

In 1994, German virologist **Stefan Lanka** raised major questions about the **very existence of all exogenous retroviruses.**

Soon afterwards, the "PG" strongly emphasized that HIV had **never** been properly **isolated**. During the international, **large** debate held at the European Parliament, in 2003, I further stressed the problems encountered in **isolating** HIV. But stressing the difficulty to **isolate** a virus remains short of stating that this virus does **not exist,** and is, consequently, **not** enough to stop all research on this virus.

As already emphasized, the appalling waste of HIV research funds must stop, and these enormous research funds should, most urgently, be re-affected towards completely different, **non**-retroviral aims. This will happen only if the worldwide scientific establishment courageously faces the fact that **HIV does not exist.** The fact that HIV **does not exist** is actually not be so surprising for all those who realize that, after almost 30

years of research, **based most exclusively on a non-scientifically verified HIV hypothesis,** 1) **not** one single AIDS patient has ever been definitely **cured** by ARVs, 2) apocalyptic epidemiological predictions **never** materialized, and 3) **not** one single efficient vaccine has ever been developed…
Obviously, we were, for 30 years on the wrong track!

Conclusively:
-we all carry, in our chromosomes**????**, defective **endo**genous retroviruses (HERVs) that have interfered, most presumably, as misleading, confounding factors in AIDS research;
-"HIV" is **not** an **endo**genous retrovirus;
-"HIV", as an exogenous, AIDS causing retrovirus simply does **not** exist, and this fact should be recognized as soon as possible for a complete, radical re-distribution of AIDS research funds, worldwide.
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Past President of Rethinking Aids (2005-2008)

https://www.thetruthbarrier.com/2013/06/20/can-electron-microscopy-resolve-the-hiv-battle-an-exclusive-interview-with-em-pioneer-dr-etienne-de-harven/

https://blog.nomorefakenews.com/2020/02/04/the-chinese-virus-hiv-and-a-stranger-on-a-train/

https://blog.nomorefakenews.com/2016/08/08/what-youll-never-read-about-virus-research-fraud/

https://blog.nomorefakenews.com/2016/04/04/does-hiv-exist-an-explosive-interview/

Epidemic: quarantining real science Mar 20 by Jon Rappoport by Jon Rappoport March 20, 2020 (To join our email list, click here.) This article is about fake science and the medical professionals who are hypnotized by it. 32 years ago, just after my first book, AIDS INC., was published, I was speaking with a doctor friend, a brilliant man. He criticized my strategy of showing how NON-VIRUS factors had destroyed the immune systems of "people with AIDS." As evidence, he cited a UCLA study which had looked into the possibility that vast overuse of antibiotics was shredding the immune systems of gay men. "You see," he said, "the study found that many gay men who had been diagnosed with AIDS didn't abuse antibiotics. Therefore, those drugs couldn't be the cause of AIDS." I was shocked. I was shocked that this doctor had fallen for absolute nonsense. First of all, I had never said antibiotics were the cause of AIDS. He was confused at the starting gate. To boil it down, my argument, in the

book, was: for various specific reasons, HIV had never been proved to be the cause of what was being called AIDS. And—this was the key—"AIDS" was a label that had been placed, like an umbrella, over a whole host of diverse health conditions. At the root, AIDS was really IMMUNE SYSTEM DESTRUCTION coming from a number of different causes, depending on which group of people you were talking about. And, in New York, Los Angeles, and San Francisco, ONE OF THOSE CAUSES, IN SOME GAY MEN, was vast overuse of antibiotics. My doctor friend hadn't understood this. WHY NOT? Here is the punch line. Through his training, he had been hypnotized into thinking that AIDS was one syndrome with one basic cause. "It had to be." 99 percent-plus of all doctors in the world had also been hypnotized in exactly the same way. One label, one basic condition, one germ. AIDS couldn't actually be a whole variety of causes, all of which suppress the immune system. No, no, no. That would be heresy. The hypnosis sets up an either-or situation. "Show us the one cause of the one condition, or go away." And that is called medical science. Imagine the following: six men in New Jersey suffer from sudden bleeding. So do eight women in New Guinea. So do twelve children in Uganda. A team of virus hunters from the CDC decides that all these occurrences must be linked by a common cause. Which, of course, will turn out to be a virus. But they're wrong. Dead wrong. It's not a virus. In fact, there is no unifying "it." The six men in New

Jersey were working in a factory where leaking acid fumes were getting into their lungs and creating hemorrhages. The eight women in New Guinea were farm workers overcome by highly dangerous pesticides, and they bled. The children in Uganda had been drinking water directly connected to sewage outlets, and in the sewage there were industrial poisons, and they bled. This was not one condition. It did not have one cause. But too late—the CDC moves in, declares it's all a virus, and the name of the condition is X-32f54d. Journal articles are rushed into print. Public health officials warn that X-32f54d could spread… You get the idea. The hypnosis works. It has nothing to do with science. In the current "epidemic," we have the same old story. IT IS NOT ONE CONDITION. IT DOES NOT HAVE ONE CAUSE. What are some of the causes which can induce the general flu-like and pneumonia-like symptoms being labeled "coronavirus?" Ordinary flu. Pneumonia from different bacteria, fungi, toxic air. TB. Common colds. Allergies. In some places, perhaps the rollout of 5G technology. Toxic vaccination campaigns. Toxic medical drugs. Highly toxic and destructive antiviral drugs, given to people who are called "COV cases." Immobilization, long-term, in nursing homes for the elderly. Pesticides causing lung problems. Industrial poisons causing lung problems. People who have slight or serious congestion and are afraid they might "have the virus" and show up at hospitals. Corporate chemical dumping. Expired and

unrefrigerated medical drugs shipped to the Third World. (The mere detection of elevated body temperature during airport screenings. People who had contact with other people who have been called “coronavirus cases.” Overeager and work-harried doctors diagnosing “suspected cases.”) And so on and so forth. NOT one condition with one cause. NOT one condition. NOT one cause. Therefore, the “spread and containment of the one virus” is wrongheaded. “But…but…suppose the patient tests positive for the coronavirus? Isn’t that some kind of proof? Doesn’t a positive test connect all these people with different conditions, under one banner?” No. I have covered this in other articles. Even assuming that researchers actually discovered COV—the diagnostic test, at best, might indicate the patient has a tiny, tiny amount of COV in his body. But, in order to cause illness, he would need to have millions and millions of virus actively replicating in his body. The test has never been proved to be capable of detecting that. And on top of all this, the overwhelming percentage of “COV cases” in the world have been diagnosed WITHOUT THE DIAGNOSTIC TEST for the virus. Therefore, what we’re left with are many people, with all sorts of different conditions, caused by many different factors—irrationally collected together under one label. “But…but…what about all these people all over the world suddenly getting sick and dying?” That’s not a true picture. In many, many cases, these are people who have been getting sick and dying in the same

ways people been getting sick for a long, long time, down through history For example, TB and pneumonia. In other cases, the causes could/would be new. For instance, new pollution, a recent vaccination campaign(s), the new rollout of 5G, the accelerated use of antiviral drugs. The other new factors are the re-labeling of all these people with a novel term: “COV”; And the press coverage, and the pronouncements of governments. And then the lockdowns. And the economic warfare against the people.

Here’s what the CDC says about the test for the Coronavirus Mar 24 by Jon Rappoport Straight from the horse’s mouth—both sides by Jon Rappoport March 24, 2020 (To join our email list, click here.) —The CDC (US Centers for Disease Control) admits the coronavirus test is flawed. That’s the overview and the takeaway— As my readers know, I’ve described why the widespread diagnostic test for the coronavirus is insufficient, misleading, useless, and deceptive. That test, used all over the world where it is available, is called the PCR. It DIAGNOSES patients. “Yes, you have the virus.” “No you don’t.” A very alert reader sent me a link to a US Centers for Disease Control (CDC) document about the test. The CDC establishes the guidelines for how the test should be done, and what the results mean. Here is a CDC paragraph about results. I suggest you read it several times. “Positive [test] results are indicative of active

infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities." I'm going to blow past the blatant contradiction in that CDC paragraph and cut to the chase. The key line in that paragraph is: "The agent detected [the coronavirus] may not be the definite cause of disease." BANG. CDC: Yeah, you see, folks, ahem, the test could say the coronavirus is there in somebody's body, but the virus may not be causing disease… On one level, the CDC is admitting the test could turn up false positives: the test could SAY a patient has the coronavirus, but he really doesn't. This isn't a footnote stuck at the bottom of a report. It's right there near the top of the section about the meaning of the test. On a deeper level, the CDC is saying straight out, IF THE TEST SHOWS A CORONAVIRUS IS PRESENT, THAT DOESN'T MEAN IT'S CAUSING DISEASE. Well, yes, I've pointed out that the test has an inherent problem. At best, it might show that a virus is present in the patient's body. But the test is incapable of determining HOW MUCH virus is ACTIVELY REPLICATING in the patient's body. And why is that important? Because, to even begin to say a virus is causing actual illness in a human, there would have to be millions and millions of a virus replicating in his body—and the PCR test has never

been proven, in the real world, to be able to make such a judgment call accurately. But, if you read that CDC quote again, you'll see the CDC is ordering labs to report a positive test result to public health agencies—where it will be counted as a "coronavirus case" come hell or high water. Thank you, CDC. So very, very much. The next ship for Uranus leaves tomorrow. Pile on board and make the trip. You can run tests there to your heart's content. This link will take you to a page with a number of links. Scroll down until you reach the link titled, "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use." That's the one.

BOTH THE BEGINNING OF THE "EPIDEMIC" AND ITS END ARE MANDATED BY ARBITRARY EDICT. Not science. Not fact. In an expanding number of countries, we are looking at medical coup. Palace revolution. Media are blaring "coronavirus coverage" wall to wall and repressing the full picture of the economic war against the people, and its human cost.

Coronavirus: toxic drugs, no liability for Pharma Mar 22 by Jon Rappoport by Jon Rappoport March 22, 2020 (To join our email list, click here.) First, we have this, from the World Health Organization (WHO): "There is no specific medicine to prevent or treat coronavirus disease

(COVID-19).” Nevertheless, doctors around the world, often with the approval of their national governments, are treating many patients with experimental or “off-label” antiviral drugs. Here are some names of the medicines: Chloroquine, Remdesivir, Ribavirin, favipiravir, lopinavir; ritonavir, hydroxychloroquine, Sofosbuvir, corticosteroids, oseltamivir, zanamivir. They all have adverse effects. What to do? Answer: decide that no one who is injured by the drugs can file a suit. In America: Done. From druganddevicelawblog.com, March 18, 2020, “We Finally Have Something To Say About COVID-19”: “On March 17, 2020, the U.S. Department of Health and Human Services (“HHS”) published in the Federal Register a ‘notice of declaration’ conferring broad-based immunity from tort (including product liability) litigation for those engaging in ‘activities related to medical countermeasures against COVID-19.’ This declaration is now published at 85 Fed. Reg. 15198 (HHS March 17, 2020).” “HHS is conferring tort immunity…The immunity extends to ‘any claim of loss caused by, arising out of, relating to, or resulting from the manufacture, distribution, administration, or use of medical countermeasures’…The immunity extends not only to COVID-19-fighting drugs, but also to ‘products or technologies intended to enhance the use or effect of a drug, biological product [vaccine], or device used against the pandemic’…The only exception is for ‘willful misconduct’.” “The immunity being conferred shoves

other federal laws aside as well as preempting state law." And that takes care of that. A patient is given an antiviral drug and dies? No law suit can be filed. Anyone associated with the drug, from manufacturer down to prescribing doctor, is exempt from liability. Take one example of a drug, Chloroquine. It's approved for the treatment of malaria, and now some doctors are using it on their COVID patients. From webmd.com, here is the "side effects" section (note: once the page loads, then click on the "Side Effects" tab at the top of the page): * "Blurred vision, nausea, vomiting, abdominal cramps, headache, and diarrhea may occur. If any of these effects persist or worsen, tell your doctor or pharmacist promptly." * "Remember that your doctor has prescribed this medication because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this medication do not have serious side effects." * "Tell your doctor right away if you have any serious side effects, including: bleaching of hair color, hair loss, mental/mood changes (such as confusion, personality changes, unusual thoughts/behavior, depression), hearing changes (such as ringing in the ears, hearing loss), darkening of skin/tissue inside the mouth, worsening of skin conditions (such as dermatitis, psoriasis), signs of serious infection (such as high fever, severe chills, persistent sore throat), unusual tiredness, swelling legs/ankles, shortness of breath, pale lips/nails/skin, signs of liver disease (such as severe

stomach/abdominal pain, yellowing eyes/skin, dark urine), easy bruising/bleeding, muscle weakness, unwanted/uncontrolled movements (including tongue and face twitching).” * “This medication may rarely cause low blood sugar (hypoglycemia). Tell your doctor right away if you develop symptoms of low blood sugar, such as sudden sweating, shaking, hunger, blurred vision, dizziness, or tingling hands/feet. If you have diabetes, be sure to check your blood sugars regularly. Your doctor may need to adjust your diabetes medication.” * “Get medical help right away if you have any very serious side effects, including: severe dizziness, fainting, fast/slow/irregular heartbeat, seizures.” * “This medication may cause serious eye/vision problems. The risk for these side effects is increased with long-term use of this medication (over weeks to years) and with taking this medication in high doses. Get medical help right away if you have any symptoms of serious eye problems, including: severe vision changes (such as light flashes/streaks, difficulty reading, complete blindness).” * “A very serious allergic reaction to this drug is rare. However, get medical help right away if you notice any symptoms of a serious allergic reaction, including: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.” * “This is not a complete list of possible side effects. If you notice other effects not listed above, contact your doctor or

pharmacist.” No liability. No law suits. No problem. Except for the patient.

Epidemic: quarantining real science Mar 20 by Jon Rappoport by Jon Rappoport March 20, 2020 (To join our email list, click here.) This article is about fake science and the medical professionals who are hypnotized by it. 32 years ago, just after my first book, AIDS INC., was published, I was speaking with a doctor friend, a brilliant man. He criticized my strategy of showing how NON-VIRUS factors had destroyed the immune systems of “people with AIDS.” As evidence, he cited a UCLA study which had looked into the possibility that vast overuse of antibiotics was shredding the immune systems of gay men. “You see,” he said, “the study found that many gay men who had been diagnosed with AIDS didn’t abuse antibiotics. Therefore, those drugs couldn’t be the cause of AIDS.” I was shocked. I was shocked that this doctor had fallen for absolute nonsense. First of all, I had never said antibiotics were the cause of AIDS. He was confused at the starting gate. To boil it down, my argument, in the book, was: for various specific reasons, HIV had never been proved to be the cause of what was being called AIDS. And—this was the key—“AIDS” was a label that had been placed, like an umbrella, over a whole host of diverse health conditions. At the root, AIDS was really

IMMUNE SYSTEM DESTRUCTION coming from a number of different causes, depending on which group of people you were talking about. And, in New York, Los Angeles, and San Francisco, ONE OF THOSE CAUSES, IN SOME GAY MEN, was vast overuse of antibiotics. My doctor friend hadn't understood this. WHY NOT? Here is the punch line. Through his training, he had been hypnotized into thinking that AIDS was one syndrome with one basic cause. "It had to be." 99 percent-plus of all doctors in the world had also been hypnotized in exactly the same way. One label, one basic condition, one germ. AIDS couldn't actually be a whole variety of causes, all of which suppress the immune system. No, no, no. That would be heresy. The hypnosis sets up an either-or situation. "Show us the one cause of the one condition, or go away." And that is called medical science. Imagine the following: six men in New Jersey suffer from sudden bleeding. So do eight women in New Guinea. So do twelve children in Uganda. A team of virus hunters from the CDC decides that all these occurrences must be linked by a common cause. Which, of course, will turn out to be a virus. But they're wrong. Dead wrong. It's not a virus. In fact, there is no unifying "it." The six men in New Jersey were working in a factory where leaking acid fumes were getting into their lungs and creating hemorrhages. The eight women in New Guinea were farm workers overcome by highly dangerous pesticides, and they bled. The children in Uganda had been drinking

water directly connected to sewage outlets, and in the sewage there were industrial poisons, and they bled. This was not one condition. It did not have one cause. But too late—the CDC moves in, declares it's all a virus, and the name of the condition is X-32f54d. Journal articles are rushed into print. Public health officials warn that X-32f54d could spread… You get the idea. The hypnosis works. It has nothing to do with science. In the current "epidemic," we have the same old story. IT IS NOT ONE CONDITION. IT DOES NOT HAVE ONE CAUSE. What are some of the causes which can induce the general flu-like and pneumonia-like symptoms being labeled "coronavirus?" Ordinary flu. Pneumonia from different bacteria, fungi, toxic air. TB. Common colds. Allergies. In some places, perhaps the rollout of 5G technology. Toxic vaccination campaigns. Toxic medical drugs. Highly toxic and destructive antiviral drugs, given to people who are called "COV cases." Immobilization, long-term, in nursing homes for the elderly. Pesticides causing lung problems. Industrial poisons causing lung problems. People who have slight or serious congestion and are afraid they might "have the virus" and show up at hospitals. Corporate chemical dumping. Expired and unrefrigerated medical drugs shipped to the Third World. (The mere detection of elevated body temperature during airport screenings. People who had contact with other people who have been called "coronavirus cases." Overeager and work-harried doctors diagnosing

"suspected cases.") And so on and so forth. NOT one condition with one cause. NOT one condition. NOT one cause. Therefore, the "spread and containment of the one virus" is wrongheaded. "But…but…suppose the patient tests positive for the coronavirus? Isn't that some kind of proof? Doesn't a positive test connect all these people with different conditions, under one banner?" No. I have covered this in other articles. Even assuming that researchers actually discovered COV—the diagnostic test, at best, might indicate the patient has a tiny, tiny amount of COV in his body. But, in order to cause illness, he would need to have millions and millions of virus actively replicating in his body. The test has never been proved to be capable of detecting that. And on top of all this, the overwhelming percentage of "COV cases" in the world have been diagnosed WITHOUT THE DIAGNOSTIC TEST for the virus. Therefore, what we're left with are many people, with all sorts of different conditions, caused by many different factors—irrationally collected together under one label. "But…but…what about all these people all over the world suddenly getting sick and dying?" That's not a true picture. In many, many cases, these are people who have been getting sick and dying in the same ways people been getting sick for a long, long time, down through history For example, TB and pneumonia. In other cases, the causes could/would be new. For instance, new pollution, a recent vaccination campaign(s), the new rollout of 5G, the accelerated use of antiviral drugs. The

other new factors are the re-labeling of all these people with a novel term: “COV”; And the press coverage, and the pronouncements of governments. And then the lockdowns. And the economic warfare against the people.

HIV: A medical coder makes a startling statement Mar 20 by Jon Rappoport Is this yet one more giant AIDS scandal? by Jon Rappoport March 20, 2020 (To join our email list, click here.) A diagnosis of AIDS without documentation of an HIV test? A diagnosis of AIDS based on some OTHER disease arbitrarily called “AIDS-related?” A medical coder (name withheld) has presented an extraordinary statement to me. I hope it will stimulate other coders to come forward and report their findings. In the coder’s following text, you will read several key claims. They strongly suggest that patients can be wrongly diagnosed with AIDS, even assuming the science behind HIV is correct (a science many independent researchers reject). This coder states that diagnoses of AIDS, as they are passed down to coders by doctors, can leave a shocking evidentiary gap, a hole which coders are supposed to ignore. The coder writes: “The job of the medical coder is to take what the doctor documents on the patient’s medical record and translate the diagnosis(es) and procedures done into codes. These codes are submitted to the payor for reimbursement. ie: private insurance, government insurance, etc.” “The coder must

only code what gets documented by the medical provider. If documentation is unclear, the coder must query the provider for further information, and the medical record gets updated or amended. If something is not documented, it CANNOT be coded." "The coder has specific coding conventions and guidelines that are to be followed and they are given and broken down in the guidelines section of the coding books. This allows easy reference for the coder." "The guidelines are broken down by chapter in the code book, and offer specific coding rules for each chapter listed." "When a patient presents to the doctor, the reason for them coming to the doctor gets documented. The doctor documents exactly what they have done to the patient and any diagnosis(es), and procedures performed." "The chapter guidelines state very clearly that when the patient presents to the doctor with symptoms of an AIDS-related illness and the doctor diagnoses the patient with an AIDS-related illness, the patient will be coded with B20, AIDS, and then followed by the code for the AIDS-related illness documented." "Here is the exact guideline right out of the coding book:" "'Code only confirmed cases Code only confirmed cases of HIV infection/illness. This is an exception to the hospital inpatient guideline Section II, H. In this context, "confirmation" does not require documentation of positive serology [test] or culture for HIV; the provider's diagnostic statement that the patient is HIV positive, or has an HIV-related illness is sufficient'." "If a patient presents to the doctor office

with symptoms of an AIDS-related illness, and they have no idea they have AIDS, and the doctor diagnoses them with an AIDS-related illness, we are safe to assume and code the patient with having AIDS. We are told that there are several AIDS-related illnesses that can be assumed to be caused by AIDS." "Once the patient presents and is confirmed to have an AIDS-related illness it is assumed and coded as AIDS forever on their medical record. No serology/culture test is needed. The assumption of the AIDS-related illness is sufficient." This medical coder is asserting that, according to official guidelines, he must list a patient as having AIDS because the doctor says so. The coder does not need to see evidence of a positive HIV test. Worse yet, according to the coder, a patient can be diagnosed with AIDS merely because he has a so-called "AIDS-related disease." No HIV test required. The CDC has, in the past, assembled a long catalog of such "AIDS-related" diseases and infections. BUT ALL OF THEM are diagnosed routinely, in the population, and not called AIDS-related. If you're beginning to think an AIDS diagnosis can be entirely arbitrary, that is what this coder is implying. Here is partial CDC list of these "AIDS-related" diseases: "Lymphoma, multiple forms; Tuberculosis (TB); Candidiasis of bronchi, trachea, esophagus, or lungs; Invasive cervical cancer; Coccidioidomycosis; Cryptococcosis; Cryptosporidiosis, chronic intestinal (greater than one month's duration); Cytomegalovirus diseases (particularly retinitis) (CMV);

Herpes simplex (HSV) [under certain conditions]…; Histoplasmosis; Isosporiasis, chronic intestinal (greater than one month's duration)." There are more. Again, the coder is stating that a patient can be coded with AIDS, forever, merely because a doctor diagnoses one of the "related" diseases, with no evidence of a positive HIV test. I hope other medical coders come forward with their findings and reports.

A fiasco in the making? As the coronavirus pandemic takes hold, we are making decisions without reliable data
By JOHN P.A. IOANNIDIS MARCH 17, 2020
he current coronavirus disease, Covid-19, has been called a once-in-a-century pandemic. But it may also be a once-in-a-century evidence fiasco. At a time when everyone needs better information, from disease modelers and governments to people quarantined or just social distancing, we lack reliable evidence on how many people have been infected with SARS-CoV-2 or who continue to become infected. Better information is needed to guide decisions and actions of monumental significance and to monitor their impact. Draconian countermeasures have been adopted in many countries. If the pandemic dissipates — either on its own or because of these measures — short-term extreme social distancing and lockdowns may be bearable. How long, though, should

measures like these be continued if the pandemic churns across the globe unabated? How can policymakers tell if they are doing more good than harm? Vaccines or affordable treatments take many months (or even years) to develop and test properly. Given such timelines, the consequences of long-term lockdowns are entirely unknown. Related: We know enough now to act decisively against Covid-19. Social distancing is a good place to start The data collected so far on how many people are infected and how the epidemic is evolving are utterly unreliable. Given the limited testing to date, some deaths and probably the vast majority of infections due to SARS-CoV-2 are being missed. We don't know if we are failing to capture infections by a factor of three or 300. Three months after the outbreak emerged, most countries, including the U.S., lack the ability to test a large number of people and no countries have reliable data on the prevalence of the virus in a representative random sample of the general population. This evidence fiasco creates tremendous uncertainty about the risk of dying from Covid-19. Reported case fatality rates, like the official 3.4% rate from the World Health Organization, cause horror — and are meaningless. Patients who have been tested for SARS-CoV-2 are disproportionately those with severe symptoms and bad outcomes. As most health systems have limited testing capacity, selection bias may even worsen in the near future. The one situation where an entire, closed population was tested was the Diamond

Princess cruise ship and its quarantine passengers. The case fatality rate there was 1.0%, but this was a largely elderly population, in which the death rate from Covid-19 is much higher. Projecting the Diamond Princess mortality rate onto the age structure of the U.S. population, the death rate among people infected with Covid-19 would be 0.125%. But since this estimate is based on extremely thin data — there were just seven deaths among the 700 infected passengers and crew — the real death rate could stretch from five times lower (0.025%) to five times higher (0.625%). It is also possible that some of the passengers who were infected might die later, and that tourists may have different frequencies of chronic diseases — a risk factor for worse outcomes with SARS-CoV-2 infection — than the general population. Adding these extra sources of uncertainty, reasonable estimates for the case fatality ratio in the general U.S. population vary from 0.05% to 1%. STAT Reports: STAT's guide to interpreting clinical trial results That huge range markedly affects how severe the pandemic is and what should be done. A population-wide case fatality rate of 0.05% is lower than seasonal influenza. If that is the true rate, locking down the world with potentially tremendous social and financial consequences may be totally irrational. It's like an elephant being attacked by a house cat. Frustrated and trying to avoid the cat, the elephant accidentally jumps off a cliff and dies. Could the Covid-19 case fatality rate be that low? No, some say,

pointing to the high rate in elderly people. However, even some so-called mild or common-cold-type coronaviruses that have been known for decades can have case fatality rates as high as 8% when they infect elderly people in nursing homes. In fact, such “mild” coronaviruses infect tens of millions of people every year, and account for 3% to 11% of those hospitalized in the U.S. with lower respiratory infections each winter. These “mild” coronaviruses may be implicated in several thousands of deaths every year worldwide, though the vast majority of them are not documented with precise testing. Instead, they are lost as noise among 60 million deaths from various causes every year. Although successful surveillance systems have long existed for influenza, the disease is confirmed by a laboratory in a tiny minority of cases. In the U.S., for example, so far this season 1,073,976 specimens have been tested and 222,552 (20.7%) have tested positive for influenza. In the same period, the estimated number of influenza-like illnesses is between 36,000,000 and 51,000,000, with an estimated 22,000 to 55,000 flu deaths. Note the uncertainty about influenza-like illness deaths: a 2.5-fold range, corresponding to tens of thousands of deaths. Every year, some of these deaths are due to influenza and some to other viruses, like common-cold coronaviruses. In an autopsy series that tested for respiratory viruses in specimens from 57 elderly persons who died during the 2016 to 2017 influenza season, influenza viruses were

detected in 18% of the specimens, while any kind of respiratory virus was found in 47%. In some people who die from viral respiratory pathogens, more than one virus is found upon autopsy and bacteria are often superimposed. A positive test for coronavirus does not mean necessarily that this virus is always primarily responsible for a patient's demise. If we assume that case fatality rate among individuals infected by SARS-CoV-2 is 0.3% in the general population — a mid-range guess from my Diamond Princess analysis — and that 1% of the U.S. population gets infected (about 3.3 million people), this would translate to about 10,000 deaths. This sounds like a huge number, but it is buried within the noise of the estimate of deaths from "influenza-like illness." If we had not known about a new virus out there, and had not checked individuals with PCR tests, the number of total deaths due to "influenza-like illness" would not seem unusual this year. At most, we might have casually noted that flu this season seems to be a bit worse than average. The media coverage would have been less than for an NBA game between the two most indifferent teams. Some worry that the 68 deaths from Covid-19 in the U.S. as of March 16 will increase exponentially to 680, 6,800, 68,000, 680,000 … along with similar catastrophic patterns around the globe. Is that a realistic scenario, or bad science fiction? How can we tell at what point such a curve might stop? The most valuable piece of information for answering those

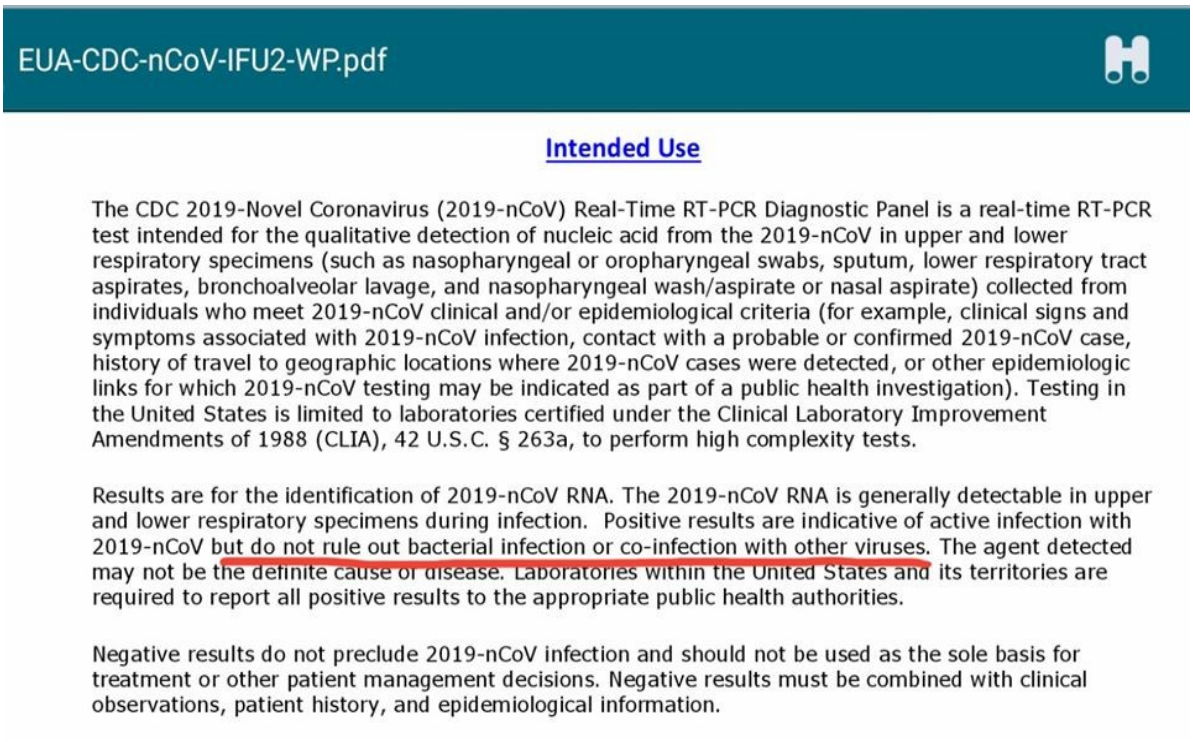
questions would be to know the current prevalence of the infection in a random sample of a population and to repeat this exercise at regular time intervals to estimate the incidence of new infections. Sadly, that's information we don't have. In the absence of data, prepare-for-the-worst reasoning leads to extreme measures of social distancing and lockdowns. Unfortunately, we do not know if these measures work. School closures, for example, may reduce transmission rates. But they may also backfire if children socialize anyhow, if school closure leads children to spend more time with susceptible elderly family members, if children at home disrupt their parents ability to work, and more. School closures may also diminish the chances of developing herd immunity in an age group that is spared serious disease. This has been the perspective behind the different stance of the United Kingdom keeping schools open, at least until as I write this. In the absence of data on the real course of the epidemic, we don't know whether this perspective was brilliant or catastrophic. Flattening the curve to avoid overwhelming the health system is conceptually sound — in theory. A visual that has become viral in media and social media shows how flattening the curve reduces the volume of the epidemic that is above the threshold of what the health system can handle at any moment. Related: The novel coronavirus is a serious threat. We need to prepare, not overreact Yet if the health system does become overwhelmed, the majority of the extra deaths may not be

due to coronavirus but to other common diseases and conditions such as heart attacks, strokes, trauma, bleeding, and the like that are not adequately treated. If the level of the epidemic does overwhelm the health system and extreme measures have only modest effectiveness, then flattening the curve may make things worse: Instead of being overwhelmed during a short, acute phase, the health system will remain overwhelmed for a more protracted period. That's another reason we need data about the exact level of the epidemic activity. One of the bottom lines is that we don't know how long social distancing measures and lockdowns can be maintained without major consequences to the economy, society, and mental health. Unpredictable evolutions may ensue, including financial crisis, unrest, civil strife, war, and a meltdown of the social fabric. At a minimum, we need unbiased prevalence and incidence data for the evolving infectious load to guide decision-making. In the most pessimistic scenario, which I do not espouse, if the new coronavirus infects 60% of the global population and 1% of the infected people die, that will translate into more than 40 million deaths globally, matching the 1918 influenza pandemic. The vast majority of this hecatomb would be people with limited life expectancies. That's in contrast to 1918, when many young people died. One can only hope that, much like in 1918, life will continue. Conversely, with lockdowns of months, if not years, life largely stops, short-term and long-term consequences are

entirely unknown, and billions, not just millions, of lives may be eventually at stake. If we decide to jump off the cliff, we need some data to inform us about the rationale of such an action and the chances of landing somewhere safe. John P.A. Ioannidis is professor of medicine and professor of epidemiology and population health, as well as professor by courtesy of biomedical data science at Stanford University School of Medicine, professor by courtesy of statistics at Stanford University School of Humanities and Sciences, and co-director of the Meta-Research Innovation Center at Stanford (METRICS) at Stanford University. About the Author John P.A. Ioannidis jioannid@stanford.edu @METRICStanford Tag

https://www.statnews.com/2020/03/17/a-fiasco-in-the-making-as-the-coronavirus-pandemic-takes-hold-we-are-making-decisions-without-reliable-data/comment-page-44/?fbclid=IwAR3721yykyEXwhM-bezVJ9O7FVrOUX0kftH8lX8W9PDAVKqLwvcJ3LkSjZU#comments

corona is a FAKE virus . cold season . many fall sick . declare that to be corona . you get a pandemic . 5g -- i havent studied . tamiflu creates flu like symptoms. give tamilfu to too many . get a real pandemic .



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Intended Use

The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals who meet 2019-nCoV clinical and/or epidemiological criteria (for example, clinical signs and symptoms associated with 2019-nCoV infection, contact with a probable or confirmed 2019-nCoV case, history of travel to geographic locations where 2019-nCoV cases were detected, or other epidemiologic links for which 2019-nCoV testing may be indicated as part of a public health investigation). Testing in the United States is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in upper and lower respiratory specimens during infection. Positive results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

remarks by a federal health official on Tuesday appeared to suggest that the World Health Organization's diagnostic tests were wildly **inaccurate**. In a somewhat rambling answer to a question related to W.H.O. tests, Dr. Deborah Birx, the White House coronavirus response coordinator, said: "It doesn't help to put out a test where 50 percent or 47 percent were **false positives.**

she was referring to a study of an early diagnostic test used in **China**. The paper found that, in a specific subset of those tested in China — asymptomatic contacts of known cases — the tests wrongly found them to be positive 47 percent of the time.

Differing diagnostic tests are now made by state laboratories, medical school laboratories and private companies like Thermo Fisher, which she mentioned as an example.

Dr. Birx said she was strongly urging commercial providers to get their tests out, but of course, they first had to prove to the Food and Drug Administration that they were of high quality. Later, she was asked about a **criticism made by former Vice President Joseph R. Biden Jr.** in Monday's night's debate. He said the W.H.O. had "offered tests to the United States but we didn't buy them."

In her answer, she did not refer to the W.H.O. tests at all, but said, "We don't buy tests that haven't been quality-controlled and they show us the data," then adding that a **test with high rates of inaccuracy would be a disaster.**

The accuracy of the test was validated by **three** laboratories before it was rolled out, the spokeswoman said, and it had consistently showed "good performance in laboratory and clinical use, and **neither a significant number of** false positive nor false negative results have been reported."

The W.H.O. does not sell tests to wealthy countries, which usually prefer to make their own. Dr. Anne Schuchat, deputy principal director of the Centers for Disease Control and Prevention, confirmed that the W.H.O. gave test kits **"primarily to under resourced** countries." Another administration official, speaking on

the condition of anonymity, confirmed that the W.H.O. had **never** offered to sell or give tests to the United States.

China, Hong Kong, France, Germany, Thailand and the United States have **all designed their own tests,** according to the W.H.O. website. Each one looks for the presence of two or three short stretches of viral genes. For example, the C.D.C's test looks at **three targets on** the N gene, while the tests ordered by the W.H.O. look **at bits of the** N gene, the RdRP gene and the E gene. Each gene performs a different function in helping the virus break into cells, hijack their DNA machinery and reproduce million of copies of itself. For countries that are unable to make the tests or buy them from other countries, the W.H.O. asks **academic or government laboratories to make tests.** It then delivers them to poor and middle-income countries at low or no cost, paying for them out of emergency funds or loans from institutions like the **World Bank.**

The test ordered by the W.H.O. was designed in a lab run by **Dr. Christian Drosten** at the medical school of Berlin's Charity Hospital, which is considered one of the world's top genomic laboratories.
Christian Drosten, director of the Institute for Virology at Charity Hospital in Berlin, in January.

According to a detailed description of the test posted on the W.H.O. website, in its initial rollout, **it was accurate 100 percent of the time.**?????

In a Feb. 21 email, another W.H.O. spokesman said the **test's accuracy had been verified by three other** laboratories before it was sent to a German diagnostics company for manufacturing. There had been no problems with the first shipment of 250,000 doses, he said.
Dr. Michael Mina, an assistant professor of epidemiology at the Harvard School of Public Health, said both the W.H.O. test and the initial C.D.C. tests were "exceptional" in their accuracy. **The problems with the C.D.C. test have been attributed to flaws in the manufacturing of reagents for kits, not in the** C.D.C.'s design. **No test is accurate 100 percent of the time, but the errors are usually introduced by medical personnel who fail to take samples correctly or lab personnel who run the test incorrectly or accidentally contaminate it** with stray DNA. For example, in February an American passenger released from the cruise ship Westerdam, which went from port to port for many days before Cambodia allowed it to dock, tested positive for the virus as she passed through Malaysia, setting off a crisis.
The C.D.C. later said she did not have the virus and judged the Malaysian test to be a **likely false positive.** Since Malaysia did not have its own test, it presumably

used the W.H.O.'s. But Malaysia **does not have a top-quality lab,** and many labs make initial errors when they are rolling out a new test.
https://www.nytimes.com/2020/03/17/health/coronavirus-tests-who.html

Rapid, Portable Tests for Coronavirus Here's something that sounds decidedly useful right now: A home test kit that anyone could use to see if they have the coronavirus. A kit that's nearly as easy to use as a pregnancy test, and that would give results in half an hour. **It doesn't exist yet, but serial** biotech entrepreneur **Jonathan Rothberg** is working on it. ((Jonathan Marc Rothberg .. In September, 2018, Butterfly Network raised $250 million from investors Fidelity, the **Gates** Foundation, and Fosun Pharma at an estimated $1.25 billion valuation.)).We've heard from Rothberg before. Over the past two decades, he has founded several genetic sequencing companies that introduced breakthrough technologies, making genome sequencing faster and cheaper. (With one company he made news by sequencing the first individual human genome, that of Jim Watson; he sold another company for US $375 million.) In 2014, Rothberg launched a startup accelerator called 4Catalyzer dedicated to the invention of smart medical devices. The first company to emerge from the accelerator, Butterfly Network, now sells a handheld ultrasound tool that provides **readouts** on a smartphone screen. Rothberg announced his interest in a **coronavirus**

test kit in a Twitter thread on 7 March. Initially describing it as a **"thought experiment,"** it quickly became a real project and a driving mission. "I told my team that the Chinese built a hospital in Wuhan in 10 days so we should be able to develop and deploy a true **home test for** Covid19 coronavirus in that time," Rothberg tells IEEE Spectrum in an email. "The team is working around the clock to accomplish that goal." Rothberg says he's having discussions with the **Gates** Foundation, which has **backed some of his other** companies and projects. The foundation has embarked on its own effort for at-home coronavirus testing, and Rothberg says the two approaches are complementary. The **Gates Foundation's kits** include a nasal swab that people mail back to the lab for analysis on "gold standard" machines, giving them results in a few days. Rothberg's kits would give results on a smartphone **in half an hour.** However, as he noted in his Twitter comments, his test **would be less accurate,** occasionally giving both false positive **and** false negative results. If the two projects share data, Rothberg says, his team could compare results and get a better understanding of their own kit's accuracy. **People who start with Rothberg's kit could also follow up with a Gates Foundation kit to confirm their results.** Rothberg says that researchers from his team and the **Gates** team are "trying hard to coordinate." And his team has already benefitted from the conversations, he says: "**Gates gave us great advice on making our kit easy, and lots of**

advice on making sure our app guides the user." Rothberg adds in his email that the **two** organizations have complementary skill sets: "We know how to make kits and **amplify** DNA :) , they **know** epidemiology." Rothberg's contact at the foundation **couldn't** be reached for confirmation before press time. On Rothberg's side, the work is being led by scientists at Homodeus, a **synthetic** biology company within the 4Catalyzer accelerator. Here's **how** Homodeus's home kit will **theoretically** work. A person will first use a swab to take a sample of a few cells from the inside of their nose or mouth. They'll dip that swab in a sequence of three tubes, following instructions from the accompanying **app**. Within each of those three tubes, **chemical** reactions will take place (more on those below). The **color** of the liquid inside the third tube **provides** answers—it will turn one color (perhaps red) if the process has revealed the presence of the virus, and another color (perhaps green) if the person is in the clear. If the test has **mal**functioned, it won't change color at all??. The person can use their phone's camera to take a photo of the tube, and the app will give them the results and provide more information. The test will **not** detect **anti**bodies, the body's natural defenses that are marshaled to fight the coronavirus. Rothberg says his team decided **against** that approach because it can take **a few days** for an infected person to generate enough antibodies to register on a test. He wants a test that would detect the **virus immediately**, as soon as

a person contracts the **virus**. He also wanted a test that an infected person could take during their **recovery** to determine when their body had successfully cleared the **virus**. For that kind of instant detection, the test must recognize the **virus's** genetic**????** material. The current coronavirus, officially known as SARS-CoV-2, is made of a **single** strand of RNA. Now back to those tubes and the **chemical** reactions therein. In the kit's **first** tube, the cells on the swab are **broken** apart so the genetic material inside is accessible. In the **second** tube, a molecule called a **primer** looks for the unique **code** of the virus's RNA. If the primer does discover the coronavirus's RNA, **another** process begins that adds a **second** complementary DNA strand to the RNA, **making** a double-stranded molecule. The **big** advantage of this reaction is that DNA molecules are **more stable** than RNA, and it's easy to make lots of **copies** of them. Then the swab goes to the **third** tube, where **customized enzymes** will make **many** copies of the DNA molecule, making it easy to detect. Another set of enzymes in the **third** tube will handle the **color** change: One color if the virus's genetic material did start this **series** of reactions, another color if it wasn't present and no reactions occurred. "No lab. No technician. No expensive machines. No wait. **The designer enzymes do all the work."** —Jonathan Rothberg, 4Catalyzer Rothberg explains that before the coronavirus outbreak, the Homodeus scientists were **engineering** enzymes to do things like repair genes and **eat** plastics.

Now they're making **designer** enzymes that **"do the entire test** that normally takes laboratory technicians and special equipment," Rothberg says. "No lab. No technician. No expensive machines. No wait. The designer enzymes do all the work."

The **app** that will accompany the test kit will not only guide the user through the testing process, **but also automatically submit results to public health authorities.**

Eric Kabrams, the 4Catalyzer engineer who's leading work on that **app**, says that data will be shared in a way that complies with strict privacy rules for people's medical data. "Thankfully, the best practices from modern cryptography and data privacy enable solutions that balance these dual objectives of minimizing the virus' spread and protecting sensitive information," he tells Spectrum in an email. If Rothberg's team succeeds in creating this test kit, the next step will be to get it to academic labs for **validation???**. Rothberg says he expects to have prototypes to send to labs in the next few weeks. "We are already in discussions with Penn and **Yale** to verify the test at their hospitals and clinical sites as quickly as possible," he says. The researchers that Rothberg has been speaking with at the University of Pennsylvania and Yale University medical schools

couldn't be reached for comment before deadline. For an outside perspective and reality check, let's hear from **Jacqueline Linnes,** an assistant professor of biomedical engineering at Purdue University. She has **developed** a handheld **paper** device that could be used to detect **viruses**, including the **novel** coronavirus. "Since people would use this at home, the tests need to be especially reliable. [These] tests need to be virtually impossible to mess up." —Jacqueline Linnes, Purdue University.

Linnes says that the reactions Rothberg describes sound like standard processes typically conducted in labs under **controlled** conditions. "It would be fantastic to be able to do this in home settings although there are a few considerations to make sure the tests work properly for reliable results," she tells Spectrum via email. "Since people would use this at home, the tests need to be especially reliable. Out-of-laboratory waived tests need to be virtually impossible to mess up." Linnes adds that the **two-color** system would be very helpful for nervous home users, since the colors enable users to differentiate between positive, negative, and test failure results. As for the considerations, she lists a few. If the tubes aren't sealed, there's a possibility of sample contamination which could interfere with the results. She also notes that users would have to be careful with the timing: "The enzymes are **eager** to do their jobs so they will sometimes **non**-specifically amplify DNA," she says. "Especially if

the reaction runs for **too long**, **even a negative sample can appear positive."** Finally, she says that the test's validators will have to ensure that the test is sensitive enough to screen the general public—if it's only able to detect the **high** concentrations of the virus found in hospitalized patients, it wouldn't**??** be much help. Rothberg acknowledges that his team has a big technical challenge: "making this easy enough for people not to mess up." But if they do succeed in making an easy-to-use test that passes expert scrutiny**???**, it will be time to produce it in massive quantities. Rothberg says he's in discussions with manufacturers that could **rapidly** ramp up production, and that his other companies have existing distribution channels that could help get the tests out quickly. He aims "to make this test **widely available across the globe**," he says. When asked for a price estimate, he says that, with support from sponsoring organizations, he expects it to be **free for anyone who can't afford it.** Besides making the kit, validating it, and manufacturing it, Rothberg's team also has to think through the regulatory requirements. On Twitter, Rothberg said **he would seek to get his test kit approved as a "risk assessment" tool, rather than a diagnostic tool that the FDA would classify as a medical device.** He tells Spectrum that he expects the FDA to work with him, given the urgency of the situation. "I expect **no** insurmountable regulatory hurdles," he says, "because we will validate at **universities** over next few weeks and use

manufactures that practice and pass regulated manufacturing requirements." So when could this at-home test kit be in your home? "With miracles on our side—**weeks to months** if we get technical breaks and more molecular biology **volunteers** to come this weekend to Guilford [Connecticut] and help," Rothberg says. And he has a message for any **potential** volunteers brave enough to leave their quarantines: "We have guest houses and food."
https://spectrum.ieee.org/the-human-os/biomedical/diagnostics/biotech-pioneer-home-test-kit-coronavirus

Faulty COVID-19 Test Kits Tilt Scales in Coronavirus's Favor
Flawed?? coronavirus test kits from the Centers for Disease Control severely limited the number of U.S. patients screened?? in January and February.
Get ready for a lot more **confirmed??** COVID-19 diagnoses as the testing backlog is cleared.

What Went Wrong with Coronavirus Testing in the U.S.
By Robert P. Baird March 16, 2020
Inside each box were four vials, packed in stiff gray foam, which held the necessary materials, known as **reagents**, to run tests on about three hundred people. Before a state or local lab could use the C.D.C.-developed tests on actual patients, however, it had to insure that they worked the same way they had in Atlanta, a process **known as**

verification. The first batch of kits, sent to more than fifty state and local public-health labs, arrived on February 7th. Of the labs that received tests, around six to eight were able to verify that they worked as intended. But a **larger** number, about thirty-six of them, received **inconclusive** results from **one** of the reagents. **Another** five, including the New York City and New York State labs, had **problems** with two reagents. On February 8th, **several** labs reported their problems to the C.D.C. In a briefing a few days later, Nancy Messonnier, the director of the National Center for Immunization and Respiratory Diseases, said that **although** "we hoped that everything would go smoothly as we rushed through this," the **verification** problems were "part of the normal procedures." In the meantime, she said, **until new reagents** could be manufactured, all covid-19 testing in the United States would continue to take place exclusively at the C.D.C. The public-health-laboratory network was **never** intended to provide **widespread** testing in the event of a pandemic. To offer tests to anyone who wanted them, as President Trump did, on March 6th, was always going to require commercial testing facilities to come on line. Still, the three-week delay caused by the C.D.C.'s failure to get working test kits into the hands of the public-health labs came at a crucial time. In the early stages of an outbreak, contact tracing, isolation, and individual quarantines are regularly deployed to contain the spread of a disease. But these tools are **useless if suspected cases**

of a disease cannot be tested. The void **created** by the C.D.C.'s **faulty** tests made it impossible for public-health authorities to get an accurate picture of how far and how fast the disease was spreading. In **hotspots** like Seattle, and probably elsewhere, covid-19 spread undetected for several weeks, which in turn only multiplied the need for more tests. "Once you're behind the eight ball, it's very hard to catch up," Alberto Gutierrez, the former head of the F.D.A. Office of In Vitro Diagnostics and Radiological Health, which regulates tests, told me. "The problem was that containment was not done very well. At this point, we're looking at **exponential** growth, and we need to figure out how to meet an exponential demand." The covid-19 tests use polymerase chain reaction, or PCR. PCR is **highly sensitive to contamination** and other faults, which is why the **verification** step is necessary to insure accurate results. And yet while the **reagent** problems were, in their way, a fairly ordinary technical hiccup—Messonnier, at the C.D.C., was **not** spinning the situation—the cascading effects that they've had on the country's covid-19 preparations suggest a much larger problem with the way the United States has structured its pandemic response. ..According to Becker, about **five thousand virology labs in the country,** including the one at the University of Washington, met the criterion.
https://www.newyorker.com/news/news-desk/what-went-wrong-with-coronavirus-testing-in-the-us

Top Ten Most Common Real-Time qRT-PCR Pitfalls.

1 Poor Primer and Probe Design

For the most efficient design of PCR primer and probe sets for real-time qRT-PCR, we strongly recommend using primer design **software**. Most primer design **programs** include **adjustable** parameters for optimal primer and probe design. These parameters consider primer/probe Tm, complementarity, **and** secondary structure as well as amplicon size and other important factors. **Restricting** the number of identical nucleotide runs is also recommended. When designing amplicons in eukaryotic targets, choose PCR primers that span at least one exon-exon junction in the target mRNA to prevent amplification of the target from contaminating genomic DNA.

2 Using Poor Quality RNA

Degraded or impure RNA can limit the efficiency of the RT reaction and reduce yield. RNA should either be prepared from **fresh** tissue, or from tissue **treated** with an RNA **stabilization** solution such as RNA*later*® short (70–250 bp). As a result, some **degradation** of the RNA can be **tolerated**. If it is not possible to use completely intact RNA, design primers to anneal to an internal region of the gene of interest. Note that for truly quantitative RT-

PCR, partially degraded RNA may/ will **not** give an accurate representation of gene expression.

Not Using “Master Mixes”

qRT-PCR is a highly sensitive tool for analyzing RNA. As the PCR amplifies the target, errors are simultaneously amplified. Therefore, variability should be kept to a minimum whenever possible. A "**master** mix", or mixture of the reaction reagents, should be used when setting up multiple reactions to minimize sample-to-sample and well-to-well variation and improve reproducibility. To further reduce well-to-well variation, a reference dye such as ROX can be added to the master mix

Introducing Cross-Contamination

All surfaces in the PCR area should be routinely decontaminated to prevent cross contamination use of a DNA decontamination solution, such as DNA*zap*™, that **destroys** DNA, is recommended. A "No Template Control" (NTC) should be run to rule out cross contamination of reagents and surfaces. The NTC includes all of the RT-PCR reagents except the RNA template. Typically the RNA is simply **substituted** with nuclease-free **water**. No product should be synthesized in the NTC; if a product is amplified, it indicates that one or more of the RT-PCR reagents is contaminated with the amplicon

Not Using a "– RT" Control

It is virtually **impossible** to completely eliminate genomic DNA from RNA preparations. Therefore, it is important to include a **minus-reverse** transcriptase control ("No Amplification Control" or NAC) in qRT-PCR experiments. Typically, the NAC is a **mock** reverse transcription containing all the RT-PCR **reagents**, except the reverse transcriptase. If a product is seen in the NAC, it probably indicates that contaminating DNA is present in the sample

Using an Inappropriate Normalization Control

The reliability of any qRT-PCR experiment can be improved by including an invariant endogenous control in the assay to correct for sample to sample variations in qRT-PCR efficiency and errors in sample quantitation. The expression level of a good control should not vary across the samples being analyzed. 18S rRNA is often used as a **control** because it is less variant in expression level than other traditional internal controls such as ß-actin or GAPDH.

Dissociation (Melting) Curves Are Not Performed When Using SYBR® Green

Ideally, the experimental samples should yield a sharp peak (first derivative plot) at the melting temperature of the amplicon, whereas the NAC and NTC will not generate significant **fluorescent** signal. This result indicates**???** that the products are specific, and that SYBR Green I fluorescence is a **direct???** measure of

accumulation of the product of interest. If the dissociation curve reveals a series of peaks, it indicates that there is not enough discrimination between specific and **non**-specific reaction products. To obtain meaningful data, optimization of the qRT-PCR would be necessary.

Not Setting the Baseline and Threshold Properly

To obtain accurate C_t values the baseline needs to be set two cycles earlier than the C_t value for the most abundant sample. For real-time qRT-PCR data to be meaningful, the **threshold** should be set when the product is in exponential phase. Typically this is set at least 10 standard deviations from of the baseline.

The Efficiency of the Reaction is Poor

The efficiency(Eff) of the reaction can be **calculated???** by the following equation:

$$\text{Eff} = 10^{(-1/\text{slope})} - 1$$

The efficiency of the PCR should be 90–110% (3.6 > slope > 3.1), A number of **variables** can affect the efficiency of the PCR. These factors can include length of the amplicon, secondary structure, and primer design, to name a few. Although valid data can be obtained that fall **outside** of the efficiency range, the qRT-PCR should be further optimized or alternative amplicons designed.

Using an Inappropriate Range for Standard Curves

Standard curves should be prepared for each **gene??** under study for RNA quantitation (absolute or relative quantitation), or for verification of the efficiencies of the reactions for comparative quantitation (delta-delta-Ct). The standard curve should extend above and below the expected abundance of your target. Additional input quantities can be included such as the minimum and maximum RNA amounts above and below the limit of detection to help differentiate between specific and **non**-specific products
https://www.thermofisher.com/in/en/home/references/ambion-tech-support/rtpcr-analysis/general-articles/ten-most-common-real-time-qrt-pcr-pitfalls.html

Doctors in Hubei recently started diagnosing COVID-19 **clinically** based on patients' **symptoms** and lung imaging. These cases are reflected **in** the global tally of infected individuals. **Clinically**-diagnosed cases account for the approximately 15,000 new cases reported by China last week. The study authors note typical **CT** findings can help medical personnel with early screening of suspected cases. Lung **imaging** may also help **predict potential** severe complications of the illness.
How Many People Really ARE Infected? Many factors are likely to confound the real number of those who have contracted or died from SARS-CoV-2. The inclusion of clinically diagnosed cases of COVID-19 may **further** muddle the issue. Professor Paul Hunter of the University

of East Anglia told Science Media Centre that previously suspected cases of the illness are **now** considered confirmed cases **even though** some may be caused by illnesses **other than** COVID-19.
Translation: Clinical diagnosis may lead to **overdiagnosis** and misdiagnosis in some cases. Professor Hunter calls for consistency in case of definitions. That is what is needed to get an accurate picture of the extent of the outbreak and the true number of those who have been infected or died. Accurate**???** numbers also help determine the potential danger for the rest of the world. Insufficient test kits, **inaccurate** test kits, **changing definitions of what constitutes a confirmed case of** COVID-19, and overdiagnosis **and** misdiagnosis of the illness make it difficult to determine the real number of those affected. Accurate diagnosis is necessary so that hospitals and resources are allocated to real**???** cases.

Monitor for Symptoms MedicineNet author Charles Patrick Davis, MD, PhD said COVID-19 **???** causes flu-**like** symptoms that worsen to fever, coughing, and shortness of breath. "Complications may include high fever, severe cough, difficulty breathing, pneumonia, **organ** failure, and death," he states. "People may prevent or lower the risk of this viral infection by good hygiene, avoiding contact with infected people, not going into an outbreak area, and by leaving an outbreak zone," Dr. Davis concludes. Anyone who has **flu-like** symptoms

should reach out to their health care team for proper evaluation, diagnosis, and treatment.

The coronavirus is a **big family of pathogens.???** Some of them cause mild illnesses like the common cold. Others can cause fatal infections. **A coronavirus gets its name from how it looks. Under an electron microscope, these pathogens exhibit spikes that resemble the angles of a crown.** There are many coronaviruses that only infect **animals**. Some evolve in their animal hosts to infect humans. The type that infects humans was **first** identified in the **1960s.??????????** Since then, **seven** human-infecting types of coronavirus have been identified, including the **new** Coronavirus also known as COVID-2019.

On Jan. 7, 2020, Chinese health authorities announced that they had **isolated** the virus spreading in Wuhan. According to the CDC, some infected people have **few or no** symptoms, whereas others may be severely ill or **die** from the disease. ??

Is the Virus Likely to Mutate? This is **a class of virus that is known to mutate easily.????** This is a class of virus that is known to mutate easily. Prior mutations **led** to the 2002-2003 SARS outbreak, in which a virus native to civet cats mutated to spread the illness to humans. In **Saudi** Arabia in 2012, a coronavirus that infected camels mutated **to become** infectious in humans, leading to the MERS outbreak.

https://www.medicinenet.com/wuhan_coronavirus_outbreak_2019-ncov/article.htm

Unreliable real-time PCR analysis of Human **Endo**genous Retrovirus-W (HERV-W) RNA expression and DNA copy number in Multiple Sclerosis Citation metadata.. Authors: Jeremy A. Garson, Jim F. Huggett, Stephen A. Bustin, Michael W. Pfaffl, Vladimir Benes and Jo Vandesompele Date: Mar. 2009 From: AIDS Research and Human Retroviruses(Vol. 25, Issue 3) Publisher: Mary Ann Liebert, Inc. Document Type: Article Length: 1,448 words Article Preview : EDITOR: The potential role of human **endo**genous retroviruses in the pathogenesis of multiple sclerosis (MS) has been the subject of many studies since the discovery of MSRV, (1) **founder** member of the HERV-W family. (2) **Two** such studies from Prof. Power's group in Canada, (3,4) recently published in AIDS Research and Human Retroviruses, give us **serious** cause for concern. The findings of **both** studies were **based on** the use of real-time polymerase chain reaction (PCR) assays for the quantification of HERV-W RNA and DNA levels in **brain, blood, and cerebrospinal fluid** samples from patients **and** controls. We consider that **technical flaws** in the real-time PCRs employed in these studies are of **such a severe and fundamental nature that the assays are unable to generate accurate or reliable data and that the**

conclusions of the papers are therefore unlikely to be valid. One of the critical parameters used to assess the quality of real-time PCR assays is the **slope** of the regression line derived from 10-fold dilutions of calibration standards. Ideally, in a perfect PCR with 100% efficiency, the slope should be -3.32 ([2.sup.3.322] =10), but for practical purposes slopes within a range from -3.10 to -3.59 are **generally** regarded as**???** acceptable. (5) This represents an acceptable efficiency **range** of 90-110%. In view of this, we were disturbed to see slope values of -1.365 for syncytin-1 DNA and -2.276 for GAPDH DNA presented in the **legend** to Fig. 1, and -1.857 for syncytin-1 RNA in the legend to Fig. 2 of the paper by Antony et al. (3) (NB: Syncytin-1 is a member of the HERV-W family and GAPDH is a reference **gene**.) Applying the equation, E=[10.sup.(-1/slope)]-1 to the syncytin-1 DNA slope value of -1.365 gives an apparent PCR efficiency (E) of 4.4, i.e., 440%, **which is entirely implausible since** Taq polymerase cannot produce more than a doubling of the number of DNA molecules with each PCR cycle. An efficiency of 440% would **imply** a 5-fold increase in DNA with each PCR cycle, which is impossible on **theoretical** grounds. **Implausibly high apparent PCR efficiencies are a well-recognized problem,** especially with SYBR Green I quantitative real-time assays, and may be due to the generation.
https://go.gale.com/ps/anonymous?id=GALE

%7CA198414370&sid=googleScholar&v=2.1&it=r&linkaccess=fulltext&issn=08892229&p=AONE&sw=w

Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. Journal of Biomolecular Techniques, 15(3), 155-66, September 2004 Authors Stephen A Bustin - Centre for Academic Surgery Institute of Cell and Molecular Science Barts, University of London, UK. s.a.bustin@qmul.ac.uk Tania Nolan **Abstract** Polymerase chain reaction (PCR)-based assays can target either DNA (the genome) or RNA (the transcriptome). Targeting the genome generates robust data that are informative and, most importantly, generally applicable. This is because the information contained within the **genome** is context-**in**dependent; i.e., generally, every normal cell contains the same DNA sequence--the same mutations and polymorphisms. The transcriptome, on the other hand, is context-dependent; i.e., the mRNA complement and level varies with physiology, pathology, or development. This makes the information contained within the transcriptome intrinsically **flexible and variable.** If this **variability** is combined with the technical **limitations** inherent in any reverse-transcription (RT)-PCR assay, **it can be difficult to achieve not just a technically accurate but a biologically relevant result.** Template **quality**, operator **variability**, the RT **step** itself, and **subjectivity** in data analysis **and** reporting are just a few technical aspects that make real-time RT-PCR appear

to be a fragile assay that makes **accurate data interpretation difficult.** There can be little doubt that in the future, transcriptome-based analysis will become a routine technique. However, **for the time being it remains a research tool, and it is important to recognize the considerable pitfalls associated with transcriptome analysis,** with the successful application of RTPCR depending on careful experimental design, application, and validation.
https://app.dimensions.ai/details/publication/pub.1076883946

In **1977**, RNA levels were first routinely quantified via **northern** blotting
Northern blotting uses **denaturing** gel electrophoresis blotting **with** labeled DNA probes, **extensive** washing steps, followed by **multiple** film exposures to insure that an exposure within the linear range of the film is achieved
Northern blotting is relatively **complex and time consuming and requires a large** amount of RNA, making the examination of many different transcripts **difficult.** Additionally, northern blotting is **poor** at detecting low abundance RNA species.
With the discovery of reverse transcriptase, which converts RNA to DNA, PCR could be used to amplify very low levels of RNAs (reverse transcriptase PCR or RT-PCR). RNA could be converted by reverse transcriptase into a cDNA in one step and then PCR could

amplify the cDNA. With **minor** modifications, this technique can be **made** real time, allowing the comparison of the relative abundance of different RNA species. Compared to northern blotting, RT-PCR has several advantages: It requires little post-PCR processing, unlike the cumbersome multisteps of northern blotting It can analyze a wide range (> 10^7 fold) of difference in RNA quantities, unlike northern blotting. The assay is far more quantitative than northern blotting, allowing more accurate measurements of RNA species amounts.

Real-Time PCR has become an increasingly popular technique for analysis of gene expression. There are two primary methods of real-time PCR that can be performed. The first involves including the reverse transcriptase step in the same tube as the PCR reaction (**one-step**). The second method involves creating cDNA first by means of a separate reverse transcription reaction and then adding the cDNA to the PCR reaction (**two-step**). There are advantages and **disadvantages** to **both** systems that you should consider before choosing the best one for your application, these include the ease of use and cost of reaction to the resulting yield and sequence representation. One-step reactions are certainly easier to set up with less overall hands-on time, but do not provide the flexibility and control that is possible with two-step reactions.

One-step real-time RT-PCR - ideal for high throughput screening

Advantages

Accurate representation of target copy number
Simple and rapid
Fewer pipetting steps (reducing possible errors and contamination)
Best option for high-throughput screening
Best method when only a few assays are run repeatedly
Multiplex PCR of gene of interest and control can be done in single well, from same RNA sample
Gene-specific primers are used for generating the cDNA and for subsequent amplification in one tube reducing experimental variation since both enzymatic reactions take place under the same conditions, making one-step real-time RT-PCR highly reproducible. There are several other advantages of one-step reactions, these include limited sample handling and reduced bench time, which helps to decrease chances for pipetting errors and cross contamination between RT and real-time PCR steps. This method is quick to set up and makes processing multiple RNA samples easy (especially when using liquid handling robotics), when you are amplifying only a few genes of interest. It is therefore ideal for high throughput screening laboratories where only a few assays are run repeatedly, using well-established reaction conditions, with the added advantage that multiplex PCR of the gene of interest and

control genes can be done in single well, from same RNA sample.

Considerations

Usually **less sensitive as it is** impossible to optimize the two reactions separately.
Difficult to troubleshoot RT step
No stock of cDNA
With one-step real-time RT-PCR, the **quality** of the RNA used in the reaction is very important, as all of the cDNA is used for the **subsequent** PCR step, also the reaction conditions needed to support both the RT and PCR may **not** be optimal for either reaction, affect efficiency and yield. Because of this, one-step reactions may require substantially more RNA in your initial samples if you are performing multiple amplifications and variation between these different RT reactions can complicate assay interpretation significantly. One-step real-time RT-PCR is therefore generally less sensitive than two-step RT-PCR. One-step real-time RT-PCR also requires careful **evaluation** to prevent primer dimer formation because the primers will be present during the **lower** temperature conditions of the RT reaction **as well as** the PCR cycling.

Two-step real-time PCR – flexible

Advantages

Two buffers optimized for independent RT and real-time PCR. Highly sensitive. Potentially more efficient because

random primers and oligo d(T) can be used. Possibility to stock cDNA to quantify several targets. Recommended when the reaction is performed with a limiting amount of starting material.

Considerations

Time consuming

More pipetting steps (increases possible error and contamination)

Requires more optimization

With two-step real-time PCR, the use of **several** tubes means that it is more time consuming and less adaptable to liquid handling robotics and so more difficult to adopt for high throughput screening assays. The use of several tubes and pipetting steps also **exposes** the reaction to a greater risk of DNA **contamination**

In summary

Using gene specific primers, one-step real-time RT-PCR such as the SensiFAST™ One-Step kits offer a quick and simple method to detect mRNA and so are useful when analyzing a few genes over a large number of samples as less pipetting and sample manipulation reduces variation and potential **contamination**. However reaction conditions needed to support both the RT and PCR may **not** be optimal for either reaction and it is **not** possible to archive the cDNA produced during the reverse transcription reaction.

Two-step real-time RT-PCR such as the SensiFAST™ kits in contrast, offers a truly accurate determination of

mRNA and is useful when analyzing a large number of transcripts over a few samples. SensiFAST™ kits have flexibility in the priming strategy, allowing for oligo-dT, random primers or gene specific primers and are generally more sensitive than one-step as the RT and PCR occur separately and can be optimized individually. Also, the cDNA produced is more stable than the initial RNA sample and can be more easily archived for future use. https://www.bioline.com/one-step-vs-two-step-real-time-pcr.html

http://qpcr.gene-quantification.info/ v imp
https://www.enzolifesciences.com/science-center/technotes/2017/march/what-are-the-differences-between-pcr-rt-pcr-qpcr-and-rt-qpcr?/
https://www.future-science.com/doi/pdf/10.2144/05391RV01

CE SEMINAR APRIL 4 – ORANGE COUNTY 2. CE SEMINAR: CHIROCRUISE ATHENS – ISRAEL . 3. CORONAVIRUS – WHY AND HOW IT WILL SOON VANISH . 4. HYDROLYZED COLLAGEN – JOINT RECONSTRUCTION . 4 APRIL 2020 Atrium Hotel – John Wayne Airport Dr Tim O'Shea – – **Dr John Bergman** .

CORONAVIRUS – WHERE'S THE SCIENCE?

For those addicted to the everyday Kool-Aid of tabloid media at CNN, Washington Post, NY Times, Yahoo News, etc. you're in for a big surprise. Last month's

newsletter dealt with the historical context of our newest **Boutique** Epidemic. Don't have to be much of a fortuneteller to see all the usual signs falling into place: The fading of the illness despite desperate attempts to keep it alive. The lack of legitimate testing proving patients actually all have the same disease. The recent **funding**, which always signals the **end** of any Boutique Epidemic ($8B)[6]

FOXLIVE 29 FEB Last Saturday, 29 Feb, President Trump, Mike Pence, Alex Azar, and Toni Fauci held a press conference, carried live on Fox. [5] **If you didn't watch that show, you're not** really interested in coronavirus. The first thing we learned is that at present there are only 22 cases of the "novel" virus in the entire United States. **Let that fact sink in.** Out of almost 350 million people. .

ACKNOWLEDGING THE CURED That was the very **first** time a major news channel acknowledged that coronavirus **wasn't a very serious disease, because** the majority of cases recovered completely in a couple of weeks. Just like the flu. And the cured should then be deducted from the statistics. No one else does that. **Virtually every other story in all media for the past three** months simply **piles the cases up,** week after week, continuing to add to a growing list, making no allowance for patients that are no longer sick. Which is **99**.9% of them. This has been **a brand new trick** with this particular **Boutique** Epidemic – **disregarding the cured.**

A tipoff to the underlying agenda. **Result**: it looks as though numbers are **increasing** out of control and it's a growing global epidemic. **This deliberately misleading** tactic explains why reported numbers for coronavirus are all over the map, depending on the source. **The whole tone of** that Saturday's Fox press conference was in stark **contrast** to everyday news reports on coronavirus, since it all began. **Did** you notice that? It was night **and** day. For once here were some very informed people – heads of HHS, NIH, the President – all saying the same thing – that Americans should go back to work and not worry about it, that the risk in this country was minimal, if that. Seriously, **do you even know of one person with coronavirus? Their mood was the polar opposite of all other media stories.** Across the board the four speakers were very measured, calm, and consistent with the facts. [5] They all agreed that even though there may be more cases appearing, for the most part it's not a serious threat to public health. Mainly because it's no more serious a disease than the flu, except in the cases of the debilitated.

RE-CATEGORIZATION? No more serious than the flu? How about this: **What if all these new cases really are nothing but the flu?** Just the normal seasonal flu. Why not? **There's really no solid evidence to support otherwise.** This technique of **re-categorization is nothing new. It was used successfully in most of the recent Boutique** Epidemics, reported in the February

newsletter. [8] Oh no, you say this is a brand new virus, a "novel" virus. Really? **Prove it.**
The identification process for "new" COVID (or 2019-nCoV)virus or the newest SARS COVID19 virus – has been **ludicrously inconsistent** from the very beginning. At **every** level – federal, state, local. For the **first** month, the viral strain was not even identified. So anybody who **got sick with anything** was likely to be included, with **no testing at all. By symptoms only. That was to create a news story. There was the preconceived agenda.** Then in January, when they **finally decided** to say it was a new strain that was infecting everybody, **that's when they named it** 2019-nCoV, in which n stands for novel, lest anyone forget we're claiming this is a brand new bug. But then **politics** took over and somebody decided they **didn't** like that name. So voila – COVID.

Politics eclipses science. THE SCIENCE OF TESTING.
Now for the **bad** news. What is the test they **claim** to use to identify this new bug in a patient? The test is called PCR. This is the classic polymerase chain reaction test, invented in the 80s by Dr Kary Mullis. In 40 years doctors have **never come up with any test more accurate than this very flawed, theoretical estimate of microbial activity.** The test produces loads of false positives, **often failing to measure anything at all. No one is more critical of the test's reliability than the inventor himself.** Dr Kary Mullis, who won the Nobel prize for

inventing PCR to detect HIV, [9] explains its limitations —why the PCR is **not especially diagnostic, for HIV or for anything else:** "Quantitative PCR is an **oxymoron**. PCR is intended to identify substances qua**li**tatively, but by its **very** nature is **un**suited for estimating numbers. Although there is a common **mis**impression that the **viral-load** tests actually count the number of viruses in the blood, these tests **cannot** detect free, infectious viruses at all; they can **only** detect proteins that are **believed**, wrongly, to be **unique** to HIV. . "The tests can detect**???** genetic sequences of viruses, but **not** viruses themselves." [1] **Can't identify viruses?** Then **how** do we know all these people have the same disease, let alone the same novel disease? This means that with all these people who have **supposedly** been PCR tested for COVID, there is still **no** conclusive diagnostic evidence that they have any coronaviruses at all. **Let alone the same virus.** According to the inventor of the primary diagnostic test. (Mullis's testimony about the **limits** of PCR actually helped **acquit** OJ. Though Johnny **didn't** seem to need much help there, did he?)

LIMITS OF POLYMERASE CHAIN REACTION TEST.

PCR is **not** a test that isolates, identifies, or even detects any particular **virus**. If you're sick and have some viral fragments, the PCR test just amplifies?? those fragmented sequences millions of times, from the sample. For the more scientifically minded, here's a source that's a review

of the literature on the **weakness** and **unreliability** of the polymerase chain reaction: [**Regulatory Concerns of PCR** [2]

Yet the PCR test remains the standard that is cited by all corporate media as the means for diagnosing coronavirus all over the world. A little research shows us that any association with viral disease , from PCR is just a **theory at best – just an estimate.** **Nothing** like an exact science that says definitively Ebola or HIV virus or coronavirus is present in this patient. **Which is what everyday media and everyday science is pretending** with coronavirus, pounding it into their undiscriminating readers' heads, week after week. But wait. **The false science gets much worse than that. There's no evidence the PCR test is even being used at all!**

IDENTIFYING VIRUSES: THE MAGIC BOX.

There is a **blatantly false assumption, encouraged by pop media and pop science.** For decades, they've pretended that doctors have a magic box that can **isolate** and photograph viruses, and sequence its **exact** RNA – and then print an image of that sequence – and then do the same for viruses it finds within any sputum or blood sample sent in for testing. And that it can Print out the exact genetic sequence of the sample virus, and then lay it down alongside the sequence of the novel virus, to compare for a match-up. **A perfect match-up then is a positive test. This is purest science fiction.** Nothing

even close to such a machine exists in our dimension. Not even close. But the deception and assumption that it does exist pervades all media and "**scientific**" literature. **Even though they are forbidden to provide any specifics on such a testing process.**
Which is why the details of COVID testing is such a closely guarded secret, and why no local clinics claim to be able to perform such a test. Till recently, samples must be sent into CDC, who then provides a simple positive or negative response. **No information on the type of testing, or the printed results comparing the sequences is offered. Even the medical clinics who send in the sample must blindly trust in** the CDC's science, without question. And this is the **source** where all the online scoreboard numbers of "infected" COVID patients come from. Look at the CDC's webpage [10] **and notice the colossal lack of information on the specifics of testing. Their most closely guarded secret**. Looks like something written by L Ron Hubbard. And **this is the best science we have in order to substantiate an entire global crisis.**

CALIFORNIA "OUTBREAK".. A few days after the Fox Live press conference, corporate media reported **4** new cases of **COVID** in the Bay area. This was **predicted** by the Fox Live conference. But there's nothing to be worried about – **no** more serious than the flu. Then corporate news hysteria ramped up yet another notch.

Santa Clara County's scoreboard on its website amplified the **seriousness** of the four cases beyond all reason. A few days later, it was up to **20 "**confirmed**"** cases, just in Santa Clara County. [3] **Confirmed how? They don't say. Nobody knows.** At the same time another site – the California Dept. of Health – was suddenly claiming **60** cases statewide. [4] That's 3x as many cases as in the entire country just a few days earlier. How is that possible? How could one county in California **suddenly have 3x as many** cases as the entire United States after just a few days? It couldn't. The answer is: there are simply **no standard criteria. for counting** cases. Plus, there was an agenda across the board to magnify the seriousness of the "epidemic" by making the most provocative, **groundless** predictions for the near future. [7] But always with the requisite assurance that "Santa Clara County is doing everything possible to manage and limit the outbreak…" The standard bureaucrat slogan.

WHAT IF THERE'S NO CORONAVIRUS EPIDEMIC AT ALL:

What if there is no epidemic, no new COVID disease at all, and no legitimate testing procedure for counting the "infected"? Let's just make that hypothesis for a moment, and then try to disprove it. I started out with a little experiment. First I called Santa Clara County health department, 408) 992-4900 and after 30 minutes finally got through to someone. I asked if Santa Clara County

had **any** facility where I could refer my patients who had the flu, where they could be tested to rule out coronavirus. The answer was unequivocal – No – Santa Clara County has **no** testing facility for coronavirus. My next question was, well all these numbers of coronavirus cases on your website – **where are they coming from if you have no testing facility?** The representative told me that people had to go to their individual practitioners, at pulmonary clinics, etc. in order to be tested. And then these doctors would **voluntarily** inform the county so they could add the numbers to the scoreboard. OK, there's Brush-off #1.

THE ILLUSION OF TESTING – NO REAL MARKET.

My next question to the County was: **what kind of testing are the doctors using at these clinics?** Answer: we don't know.

See where this is going, as we start down the bureaucratic rabbit hole?

So my next step was to call local MDs, **especially pulmonary specialists** in the Bay Area. After calling more than 50 of these offices, the answer was a resounding No – 100% of the time. **No, we do not have any test that we can use to rule out coronavirus infection.** Try it! Pretty safe to assume that 50 is a sufficient number of clinics in the Bay Area to determine whether there's any clinic who offers PCR or any other

test for coronavirus. My contention is that there are no practitioners who have such a test and therefore coronavirus screening tests are simply not available in Santa Clara County. And therefore we have no idea how many cases there are. Or if there are any at all. If you then ask the clinic – **Do you know anyone who does have the test** – guess what they say. Take a guess. **They'll tell you to call the** Santa Clara County Dept. of Health – which is who told you to go to the individual clinics in the first place. There's Brushoff #2.

See the game here? Now why would all the pulmonary **specialists in one of the most densely** populated sections of the country have **no interest whatsoever in screening people for a respiratory disease being hawked by all media as the most dangerous epidemic threat we've ever seen…?** Obviously the respiratory MDs are **not** taking the outbreak very seriously – **not** much faith in its virulence. Think about it – if this epidemic were **real**, would all these doctors ignore an entire market, this huge?

CONCLUSION AND HYPOTHESIS

So a perfectly valid hypothesis might be this: the County's online scoreboard is **fraudulent**. There **are no proven cases at all.** These reported cases of so-called COVID are nothing more than the flu, **because** there is no available testing procedure in all of the Bay Area that can diagnose the disease – in an area with 5 million people.

And even if there were, the **standard test** cited in any medical reference for COVID is the RT-PCR test, for which we have seen above the evidence of its **inherent inaccuracy and lack of reliability.** By extrapolation then, it is fairly reasonable to say that the random reporting we see at work in one of the **richest**, **most populous** communities in the US is **no** different from what is going on everywhere else across the nation. Looks like it's all being **orchestrated** via the **same** corporate narrative. What we've seen in the past few months may be nothing more than the standard **re-categorization technique, traditionally used to conjure up new Boutique** Epidemics, in the absence of any truly novel disease. [8] We have to look at last year's figures. In every community, in very state, let's take a look at the annual numbers of people who got the flu, for the past 3 years. Guess what you'll find. **No** difference at all this year. So what was this all about – what was the objective from the outset? Was it merely the $8.3 billion pork barrel for local, state and federal employees to squabble over? With media's increasing stranglehold **and** censorship of oppo information now in place, this particular **Boutique** Epidemic got a little carried away.

REFERENCES 1. National Library of Medicine **Questioning the HIV-AIDS Hypothesis: 30 Years of Dissent** Front Public Health. 2014; 2: 154. www.ncbi.nlm.nih.gov/pmc/articles/PMC4172096/ 2.

Hu, Y **Regulatory Concern of Polymerase Chain Reaction (PCR) Carryover Contamination** https://www.intechopen.com/books/polymerase-chain-reaction-for-biomedical-applications/regulatory-concern-of-polymerase-chain-reaction-pcr-carryover-contamination 3.Santa Clara County Alerts – Coronavirus www.sccgov.org/sites/phd/DiseaseInformation/novel-coronavirus/Pages/home.aspx 4.California Dept of Public Health – Coronavirus Disease 2019 (COVID-19) https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Immunization/nCOV2019.aspx 5. FoxNews Live 29 Feb 2020 — News Alert press conference – The White House 6. Trump signs $8.3B coronavirus spending bill – Fox 6 Mar 2020 www.foxnews.com/politics/trump-cancels-trip-to-cdc-amid-coronavirus-outbreak 7. As the coronavirus spreads, … best-case scenario is 15 million dead and a $2.4 trillion hit to global GDP Yahoo news 5 Mar 2020 https://news.yahoo.com/coronavirus-spreads-one-study-predicts-101552222.html?soc_src=hl-viewer&soc_trk=ma 8. February Newsletter https://thedoctorwithin.com/blog/2020/02/10/newsletter-february-2020/ 9. Kary B. Mullis: Nobel Prize – https://www.nobelprize.org/prizes/chemistry/1993/mullis/facts/ 10. CDC: Testing in US https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/testing-in-us.html?CDC_AA_refVal=https%3A

%2F%2Fwww.cdc.gov%2Fcoronavirus%2F2019-ncov%2Ftesting-in-us.html

https://thedoctorwithin.com/blog/2020/03/10/newsletter-march-2020/ Dr. John Bergman

Scientists Say the COVID19 Test Kits Do Not Work, Are Worthless, and Give Impossible Results.

A pregnancy test is 99% accurate. The coronavirus spectrum test kit is 20% accurate or worse. If a test is only 20% accurate, is the better word "inaccurate"?

Please take about 1 minute to at least glance at the bold copy. My prior article got 30,000+ views and was criticized for my commentary. Here is my limited comments: The CDC and FDA both admit the COVID19 test kits suffer from false positives and false negatives. They just fail to tell you those rates. But others have revealed those rates. "the false-positive rate of positive results was 80.33%" and 85% false negative rate. The test kits don't work. If the test kits don't work, or are less reliable than a coin flip, then all the data on "who has it" is utterly meaningless and it's all a total fraud and hoax. People are still dying, but from the same illness as always: the flu. So, what follows is only exact quotes from the articles, and links. Below are 15 sources giving

commentary on the reliability of the COVID19 test kits in use.
From the maker of the test: "SARS-CoV-2 Coronavirus Multiplex RT-qPCR Kit (CD019RT)
Regulatory status: For research use only, not for use in diagnostic procedures." https://www.creative-diagnostics.com/sars-cov-2-coronavirus-multiplex-rt-qpcr-kit-277854-457.htm

"The New York SARS-CoV-2 Real-time RT-PCR Diagnostic Panel has been designed to minimize the likelihood of false positive test results. However, in the event of a false positive result, risks to patients could include the following: a recommendation for isolation of the patient, monitoring of household or other close contacts for symptoms, patient isolation that might limit contact with family or friends and may increase contact with other potentially COVID-19 patients, limits in the ability to work…"
https://www.fda.gov/media/135662/download
CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel For Emergency Use Only…
"Positive results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. "
"Negative results do not preclude 2019-nCoV infection"

“Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results.”
“The possibility of a false negative result should especially be considered if the patient’s recent exposures or clinical presentation suggest that 2019-nCoV infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If 2019-nCoV infection is still suspected, re-testing should be considered in consultation with public health authorities. “
” Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low. “
https://www.fda.gov/media/134922/download
“Should I be testing all patients for COVID-19?
Clinicians should base their decisions on whether a patient should be tested for COVID-19 on:
Signs and symptoms,
Local epidemiology, and
If the patient has had close contact with a confirmed COVID-19 patient or a history of travel from an area with sustained transmission within 14 days of symptom onset.”
https://www.cdc.gov/coronavirus/2019-ncov/lab/tool-virus-requests.html
[Potential False-Positive Rate Among the ‘Asymptomatic Infected Individuals’ in Close Contacts of COVID-19 Patients]

https://pubmed.ncbi.nlm.nih.gov/32133832/
“Results: When the infection rate of the close contacts and the sensitivity and specificity of reported results were taken as the point estimates, the positive predictive value of the active screening was only 19.67%, in contrast, the false-positive rate of positive results was 80.33%. The multivariate-probabilistic sensitivity analysis results supported the base-case findings, with a 75% probability for the false-positive rate of positive results over 47%. Conclusions: In the close contacts of COVID-19 patients, nearly half or even more of the ‘asymptomatic infected individuals’ reported in the active nucleic acid test screening might be false positives. “

[Potential false-positive rate among the ‘asymptomatic infected individuals‘ in close contacts of COVID-19 patients].
https://www.unboundmedicine.com/medline/citation/32133832/[Potential_false_positive_rate_among_the_’asymptomatic_infected_individuals’_in_close_contacts_of_COVID_19_patients]_

“Nearly 10 percent of the human genome is made of bits of virus DNA. For the most part, this viral DNA is not harmful. In some cases, scientists are finding, it actually has a beneficial impact.”

https://www.sciencedaily.com/releases/2016/11/161128151050.htm

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“For context, Goodman says a “really good” r-squared, in terms of public health data, would be a 0.7. “Anything like 0.99,” she said, “would make me think that someone is simulating data. It would mean you already know what is going to
happen.”” https://www.barrons.com/articles/chinas-economic-data-have-always-raised-questions-its-coronavirus-numbers-do-too-51581622840

” There’s no evidence the PCR test is even being used at all! “
Newsletter March 2020

“PCR basically takes a sample of your cells and amplifies any DNA to look for ‘viral sequences’, i.e. bits of non-human DNA that seem to match parts of a known viral genome.
The problem is the test is known not to work.
It uses ‘amplification’ which means taking a very very tiny amount of DNA and growing it exponentially until it can be analysed. Obviously any minute contaminations in the sample will also be amplified leading to potentially gross errors of discovery.

Additionally, it's only looking for partial viral sequences, not whole genomes, so identifying a single pathogen is next to impossible even if you ignore the other issues. The idea these kits can isolate a specific virus like COVID-19 is nonsense."
https://occamsrazorterrorevents.weebly.com/blog/coronavirus-hoax-jan-2020

"The Test is Not Binary
Tests for infections are usually reported as positive or negative (sometimes 'reactive' and 'unreactive'. One of the reasons for this is that, in many cases, multiple tests are required, and it is common to conclude that someone is infected with some negative tests and that someone is uninfected with some positive tests. The results of a complex multi-test algorithm are also usually reported as positive or negative, but interpreted by doctors and patients as infected or uninfected.
" But, in reality even individual tests are not binary, not positive or negative, but a range of numbers that are arbitrarily divided into positive on one side and negative on the other. Possibly there is a grey area that allows other factors, including the bias of the doctor or laboratory, to enter into the interpretation, or that will require further testing. "
"Positive to Negative and Back Again

The majority of the 18 patients had a positive test, followed by a negative test, followed by a positive test. Some had this several times.
If a negative test means uninfected, then this is impossible. You cannot rid yourself of the virus, and then be reinfected the next day, and then infected the day after and uninfected again.
The simplest answer to this conundrum is that negative tests do not mean uninfected. But the corollary is that positive tests do not mean infected. Which would make the test worthless."
https://www.greenmedinfo.com/blog/does-2019-coronavirus-exist

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Stanford epidemiologist warns that coronavirus crackdown is based on bad data
https://www.thecollegefix.com/stanford-epidemiologist-warns-that-coronavirus-crackdown-is-based-on-bad-data/
"If we had not known about a new virus out there, and had not checked individuals with PCR [virus] tests, the number of total deaths due to 'influenza-like illness' would not seem unusual this year."
"Patients who have been tested for SARS-CoV-2 [COVID-19] are disproportionately those with severe symptoms and bad outcomes." [That's ascertainment bias, confirmed above where the CDC says to only test sick people.]

VirusGuy:
Some notes on those test kits I saw you asking about on Twitter yesterday.
They don't do antibody tests. They do a thing called PCR testing, which basically takes a sample of your cells and amplifies any DNA to look for 'viral sequences', i.e. bits of non-human DNA that seem to match parts of a known viral genome.
The problem is the test is known to be bullshit.
It uses 'amplification' which means taking a very very tiny amount of DNA and growing it exponentially until it can be analysed. Obviously any minute contaminations in the sample will also be amplified leading to potentially gross errors of discovery.
Secondly, it's only looking for partial viral sequences, not whole genomes, so identifying a single pathogen is next to impossible even if you ignore the other issues.
All these Mickey Mouse test kits being sent out to hospitals do at best is tell the analysts you have some viral DNA in your cells. Which most of us do, most of the time. It may tell you the viral sequence is related to a specific type of virus – say the huge family of coronavirus. But that's all.
The idea these kits can isolate a specific virus like covi-19 is utter bullshit.
And that's not even getting into the other issue – viral load.

If you remember the PCR works by amplifying minute amounts of DNA. It therefore is useless at telling you how much virus you may have.

And that's the only question that really matters when it comes to diagnosing illness. Like I said, everyone will have a few virus kicking round in their system at any time, and most will not cause illness because their quantities are too small. For a virus to sicken you you need a lot of it, a massive amount of it. But PCR does not test viral load and therefore can't determine if a osteogenesis is present in sufficient quantities to sicken you.

If you feel sick and get a PCR test any random virus DNA might be identified even if they aren't at all invo lved in your sickness. Leading to false diagnosis.

And coronavirus are incredibly common. A large percentage of the world human population will have covi DNA in them in small quantities even if they are perfectly well or sick with some other pathogen.

Do you see where this is going yet?

If you want to create a totally false panic about a totally false pandemic – pick a coronavirus.

They are incredibly common and there's tons of them. A very high percentage of people sick by other means (flu, bacterial pneumonia, anything) will have a positive PCR test for covi even if you're doing them properly and ruling out contamination, simply because covis are so common. There are hundreds of thousands of flu and pneumonia

victims in hospitals throughout the world at any one time. All you need to do is select the sickest of these in a single location – say Wuhan – administer PCR tests to them and claim anyone showing viral sequences similar to a corona virus (which will inevitably be quite a few) is suffering from a ‘new’ disease.

Since you already selected the sickest flu cases a fairly high proportion of your sample will go on to die.

You can then say this ‘new’ virus has a CFR higher than the flu and use this to infuse more concern and do more tests which will of course produce more ‘cases’, which expands the testing, which produces yet more ‘cases’ and so on and so on.

Before long you have your ‘pandemic’, and all you have done is use a simple test kit trick to convert the worst flu and pneumonia cases into something new that doesn’t actually exist.

Now just run the same scam in other countries. Making sure to keep the fear message running high so that people will feel panicky and less able to think critically.

Your only problem is going to be that – due to the fact there is no actual new deadly pathogen but just regular sick people you are mislabelling – your case numbers, and especially your deaths, are going to be way too low for a real new deadly virus pandemic.

But you can stop people pointing this out in several ways.

1. You can claim this is just the beginning and more deaths are imminent. Use this as an excuse to quarantine

everyone and then claim the quarantine prevented the expected millions of dead.
2. You can tell people that ‘minimising’ the dangers is irresponsible and bully them into not talking about numbers.
3. You can talk bullshittery about r0 numbers hoping to blind people with pseudoscience
4. You can start testing well people (who of course will also likely have shreds of coronavirus DNA in them) and thus inflate your ‘case figures’ with ‘asymptomatic carriers’ (you will of course have to spin that to sound deadly even though any virologist knows the more symptomless cases you have the less deadly is your pathogen
Take these simple steps and you can have your own entirely manufactured pandemic up and running in weeks.
But why are you doing this people may ask.
Lots of reasons. Fear is useful. And a population frightened into demanding protection will accept anything you do to ‘protect’ them, up to and including nailing them into their own houses.
It can be a trial run for social control methods. To see how gullible populations are. To enforce more rigorous censorship. To inure people to shortage and uncertainty. All these things and others are reasons.
But getting hung up on possible motive misses the point – that all the evidence points to this being the case.

Everything I am seeing points at a fake manufactured pandemic. The low numbers and attempts to inflate them with scary anecdotes and bad science, the crazy overreaction in world governments, as if the reaction itself is the point. The ridiculous numbers of famous people ‘testing positive’.
It could easily be done and it looks as if it is. In my view. But you must make up your own mind.
I think many in the virology and epidemiology line would agree, but no one is going to risk their career right now saying so in public. They might as well jump off of a brid ge.
You can verify everything I have said about the PCR test.

Reported case fatality rates, like the official 3.4% rate from the World Health Organization, cause horror — and are meaningless. Patients who have been tested for SARS-CoV-2 are disproportionately those with severe symptoms and bad outcomes.

“The accuracy of the current COVID-19 tests is not precisely known**.”**
The accuracy of COVID-19 tests
RICHARD L. HUTCHISON, MD | CONDITIONS | MARCH 12, 2020
https://www.kevinmd.com/blog/2020/03/the-accuracy-of-covid-19-tests.html

” As a physician, I treat the results of lab tests like I treat movie recommendations from a friend – I am always skeptical. “
“My friend’s movie judgments are occasionally biased and off-kilter. In the same way, medical diagnostic test results are not perfect. There is always the chance that they provide incorrect information.
Medical professionals, policymakers, and members of the general public may overestimate the accuracy of diagnostic tests. The usefulness of any test depends on how likely the patient has the disease, the ability of the test to correctly identify the disease, and the capability of the test to correctly confirm the condition is not present. Unfortunately, test results will be negative for some people that actually have the disease, and some people without the disease will have positive tests.”
“ The accuracy of the current COVID-19 tests is not precisely known. Reasonable estimates, based on test performance in China and the performance of the influenza tests, are that the tests will correctly identify around 60 percent of the patients with the disease and correctly identify 90 percent of the patients that are disease-free. “
” Assume that the physician thinks there is a 50 percent of the patient having COVID-19. Given the above numbers, if the patient has the disease, the test will be positive 85 percent of the time. Fifteen percent of the infected patients will incorrectly be diagnosed as not having the

disease. If the patient does not have the disease, only 70 percent of the patients will have a negative test. It would take four consecutive negative tests to conclusively prove the patient did not have the disease. “

“There has been the worry of how effective the tests for the coronavirus has been as of late. There are numerous talks in several countries that suggest people are having over six negative results before finally being diagnosed as positive for the virus.
The question of the effectiveness of the tests because the officials in Hubei province, China, have started to COUNT people with symptoms rather than using the tests to confirm that they indeed have the coronavirus.”
https://www.techtimes.com/articles/247389/20200217/urgent-is-the-coronavirus-tests-completely-sure-or-is-it-more-of-a-hoax.htm

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“First, the prevailing diagnostic test for COVID-19 may be only 30 to 40 percent
accurate.” https://www.foxnews.com/opinion/lew-olowski-coronavirus-worse-than-reported-heres-how-china-is-making-the-situation-worse

https://revealingfraud.com/2020/03/health/test-kits-do-not-work/

https://www.creative-diagnostics.com/sars-cov-2-coronavirus-multiplex-rt-qpcr-kit-277854-457.htm

Below are a number of indicators that the alleged coronavirus pandemic is, essentially, a globally orchestrated "live exercise" in managing a pandemic (preceded in October last year by Event 201, a "pandemic tabletop exercise"). We can infer that the alleged purpose of this exercise is really a flimsy pretext for fear-mongering instigated by the global power elite in order to exercise better control - there are about 8,500 of them and 7 billion of us. The scope of social control laid bare by this pandemic is truly scary. What social controls will be implemented and how this event will be used as a pretext for blaming looming economic problems only time will tell.
https://occamsrazorterrorevents.weebly.com/blog/coronavirus-hoax-jan-2020

https://chestofbooks.com/health/children/Herbert-Shelton/The-Hygienic-Care-of-Children/index.html

The **population is constantly threatened with epidemics**.--Dr Buchwald MD

'What level of fear needs to be created to appear as convincing as possible?'"---Dr Buchwald (The Decline

of Tuberculosis **despite** "Protective" Vaccination by Dr. Gerhard Buchwald M.D. p104)

Regulatory Concern of Polymerase Chain Reaction (PCR) Carryover Contamination.. Yuan Hu

Additional information is available at the end of the chapter http://dx.doi.org/10.5772/66294

Abstract
Currently, DNA amplification techniques have become important detection tools. However, the extreme sensitivity of such techniques can easily result in contamination. This is a major problem in using these techniques routinely in a regulatory agency such as the Food and Drug Administration (FDA) because false-positive polymerase chain reaction (PCR) results will fail our mission. Preventing PCR carryover contamination and a capacity to rapidly determine false PCR positives are crucial. In the past, several methods have been used to prevent amplicon carryover contamination. In this chapter, we provide practical suggestions for PCR carryover contamination detection and prevention that work well with most PCR applications in our laboratory.
Keywords: polymerase chain reaction (PCR), carryover contamination, nested PCR, real-time PCR, single-tube nested real-time PCR 1.

Introduction

Polymerase chain reaction (PCR) amplification techniques have provided means for the rapid and sensitive detection of pathogens [1]. The number of applications of PCR is still growing, and more and more amplificationÕbased techniques are now used in FDA field laboratories to detect pathogens, such as Salmonella, Escherichia coli 0157:H7, Shigella, Vibrio, hepatitis A virus (HAV) and noroviruses (NoVs) [2]. A significant challenge facing us is that the sensitivity of PCR can easily result in contamination and consequently in false-positive PCR. A small amount of previously amplified PCR product or potential target sequences that infiltrate laboratory supplies and equipment or that are present in an aerosol can easily contaminate the sample and PCR reagents in the tests. Therefore, prevention of carryover © 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in

contamination from previous PCR amplifications has become a high priority. As a first line of defense to prevent contamination of PCR with a previously generated amplicon, mechanical separation of the PCR laboratory into different rooms or laboratory benches is needed. Secondly, chemical, UV, and enzymatic methods can be applied to inactivate any prior amplicon generated in the laboratory. Additionally, rapid identification of contaminants and their sources is needed to prevent false-positive PCR results. For this purpose, we developed a rapid method to detect PCR carryover contamination by DNA sequencing. The combination of the above methods plus good laboratory technique should be able to totally eliminate PCR carryover contamination and allow us to perform accurate and sensitive PCR routinely in regulatory settingʒ

1.1. Polymerase chain reaction

In 1983, Dr. Kary Mullis at Cetus Corporation conceived of polymerase chain reaction. There is not any technique that has had a greater impact on the practice of molecular biology than PCR. PCR-based methods are powerful techniques [3]. This technique is centered around the ability of sense and anti-sense DNA primers to hybridize to a DNA of interest. When put into use, agents of infectious diseases can be detected at extremely low levels. After extension from the primers on the DNA

template by DNA polymerase, the reaction is heat-denatured and allowed once again, to anneal with the primers. After another round of extension, a multiplicative increase in DNA products is observed. When critical controls are set, this technique becomes a quantitative process. Therefore, a minute amount of DNA can be efficiently amplified in an exponential fashion to result in an easily manipulable amount of DNA. The current sensitivity and detection limit is at a level as low as 10–50 copies per ml. Although the PCR is extremely easy and fast, PCR product carryover contamination impedes the routine use of these techniques routinely in regulatory laboratories.

1.2. PCR-based technology

1.2.1. Reverse transcription PCR (RT-PCR) PCR uses DNA as a starting material. When RT-PCR is carried out, the starting material is RNA. In this method, RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase from total RNA or messenger RNA (mRNA). The cDNA is then used as a template for the quantitative PCR (qPCR). Real-time quantitative PCR (RT-qPCR) is used in a variety of applications such as food-borne RNA virus and avian flu virus detection. With this technique, we can detect the target RNA at an extremely low level in samples. RT-PCR is an increasingly popular method for RNA virus detection, but DNA contamination in RNA preparations is also a

concern. In order to minimize the possibility of carryover contamination in RT-PCR, it is critical to minimize the number of handling and pipetting steps.
1.2.2. Real-time quantitative PCR (qPCR) Traditional detection of amplified PCR product relies upon gel electrophoresis. qPCR is an advanced form of the traditional PCR. It is a major development in PCR technology that enables 58 Polymerase Chain Reaction for Biomedical Applications the reliable detection and measurement of products generated during every cycle of the PCR process. This technique **became** possible after the introduction of an **oligonucleotide** probe that was designed to hybridize within the target sequence. Due to the 5′ nuclease activity of Taq polymerase, amplification of the targetÕspecific product can be detected through cleavage of the probe during PCR. These assays are very sensitive and can detect as few as 10–100 viral copies per reaction. qPCR techniques have evolved into a variety of other branches including real-time PCR by Taqman (Roche), LightCycler by (Roche), SmartCycler by (Cepheid), etc. Some of them are now widely in use for virus and bacteria detection in regulatory laboratories. Unlike other PCR methods, qPCR does not require post-PCR product handling, preventing potential PCR product carryover contamination. 1.3. PCR contamination All the PCR methods are **powerful???** techniques. Unfortunately, the exquisite sensitivity of these techniques makes them vulnerable to contamination [4,

5]. One of the most important rules when performing PCR is to avoid contamination. This chapter will outline necessary precautions to prevent contamination as well as procedures for detecting and cleaning suspected contamination.

2. Potential sources of contamination 2.1. Cross contamination between samples A large number of target organisms in sample handling may lead to preÕamplification sample cross contamination [6, 7]. The sources of contaminants between samples are diverse and can all contribute to the contamination of the finished PCR product. These sources may include reagents, disposable supplies, sample carryover, improper handling procedures, etc. 2.2. Cross contamination between nucleic acids Cross contamination between nucleic acids is a major problem in all PCR laboratories. Nucleic acids from organisms or plasmid clones derived from organisms that have been previously analyzed and that may be present in large numbers in the laboratory environment could be a source of contamination. Contaminants can also be introduced by unrelated activities in neighboring laboratories. These sources of contamination are problematic as they may lead to preÕamplification cross contamination [8–10]. 2.3. PCR product carryover contamination The most important source of contamination is from the repeated amplification of the same target sequence, which leads to

accumulation of amplification products in the laboratory environment. Even minute amounts of carryover can lead to false-positive results. A typical PCR generates theoretically as many as 108 copies of target sequence [11]. If uncontrolled, amplification products will contaminate laboratory reagents, equipment, and ventilation Regulatory Concern of Polymerase Chain Reaction (PCR) Carryover Contamination http://dx.doi.org/10.5772/66294 59 systems. Carryover of previously accumulated amplified DNA is considered the major source of contamination.

3. Methods to control contamination Contamination between samples and from previous PCR amplicon generation is a significant potential source of invalid PCR results [12]. The first two forms of contamination described above can be easily avoided by using careful technique and good quality control practices. Generally, most PCR-based assays consist of three steps: DNA sample processing, PCR amplificationǰ and amplification product detection (excluding real-time PCR). It is in the latter step that carryover contamination often occurs through methods that include gel electrophoresis, solid phase hybridization, solution hybridization, and capillary electrophoresis [7]. Methods to prevent amplification product carryover contamination have been developed in the past ten years [13–17]. Basically, there are mechanical, chemical, UV light irradiation, and

enzymatic methods and closed-tube PCR detection formats, all of which can help to prevent amplification product carryover contamination [7]. The following section will focus on more recent practices and methods that have been used in our laboratory to eliminate carryover contamination. 3.1. Mechanical method Our laboratory was designed and operated in a way that prevents contamination of reactions with PCR products from previous assays and cross contamination between samples. It includes the separation of areas of the laboratory where samples and reagents are prepared from the areas where amplification is performed and amplification products are analyzed. This unidirectional workflow can reduce the opportunity for contamination to occur. A typical PCR laboratory should be divided into at least three to four different areas—(1) sample preparation, (2) PCR mix preparation, (3) PCR product detection, and (4) RNase free area—if the PCR method involves RNA sample. 3.2. Chemical method General cleaning practices are important for controlling PCR carryover contamination. All surfaces in the PCR area should be routinely decontaminated to prevent cross contamination. The PCR work bench is required to be **cleaned** with 10–15 % sodium hypochlorite solution (bleach), followed by removal of the bleach with 70 % ethanol. 3.3. UV irradiation method UV irradiation is an easy method to inactivate amplification product involved in carryover contamination. The method is based on the ability of UV

light to induce thymidine dimer formation in the DNA that makes the contaminating nucleic acid inactive as a template for further amplification (Figure 1). A good practice is to expose all of the PCR supplies to UV light for 5–20 min as the nucleic acid will be damaged by absorbing the UV light energy at 60 Polymerase Chain Reaction for Biomedical Applications 254 nm wavelength [19]. UV irradiation is an integral feature of our PCR laboratory, and the Spectrolinker XL-1500 (Spectronics Corporation, Westbury, NY) is used to eliminate contamination that may occur during PCR tests. All of our PCR tools are stored in a UV light box (C.B.S Scientificj Co. Del Mar, Ca). PCR master mix preparation and specimen setup are also carried out in this UV light box.
Figure 1. Action of UV light on the nucleic acids [18].
3.4. Enzymatic method Uracil-DNA glycosylase (UNG) is a DNA repair enzyme [20] that can recognize and remove uracil residues from DNA (Figure 2). In 1990, the use of UNG to inactivate PCR products was first reported [21]. This method employs uracil (dUTP) instead of thymine (dTTP) during PCR to generate amplification products with distinguishing characteristics relative to the native DNA template. Because the newly synthesized amplicons contain dUTP, they are susceptible to hydrolysis by UNG. This method is the most widely used contamination control**???** technique in our laboratory.
Figure 2. Replace dTTP with dUTP during PCR amplification and the PCR product will contain uracil.

Prior to PCR, the PCR mixture is treated with uracil-DNA glycosylase (UNG). During the denaturation step, temperature is elevated to 95°C, resulting in cleavage of apyrimidinic sites and fragmentation of carryover DNA. As the template contains thymidine, it will not be affected by the UNG treatment (source: Sopachem Life Sciences). Regulatory Concern of Polymerase Chain Reaction (PCR) Carryover Contamination http://dx.doi.org/10.5772/66294 61 Brieflyǰ this carryover prevention technique consists of three steps: 1. The dUTP is incorporated into all PCR products, substituting dUTP for dTTP or incorporating dUTP during synthesis of the primers [15, 22]. 2. Before PCR, mixtures are treated with UNG (Applied Biosystems, Foster City, CA) at room temperature for 10 min to hydrolyze and remove any contaminating amplification products that may be present in the PCR mixtures. This technique also has a hot start function [15]. 3. UNG is thermally inactivated at 95°C for 5 min prior to the actual PCR. 3.5. Another mpl□ę□□□ □on format without the risk of carryover contamination 3.5.1. Real-time PCR-based technology to avoid contamination In traditional PCR, amplification and detection of the target DNA sequence occur separately. To determine if a sample contains the target sequence, postÕamplification handling of the amplicon is required. A more recent technological development, real-time PCR [23], allows for the simultaneous amplification and detection of a target sequence through the use of fluorescent labeled probes

(Figure 3). In comparison to conventional PCR, real-time PCR can reduce the chance of carryover contamination. The new generation of amplification technology simultaneously amplifies and detects target DNA without exposing the amplification products to the laboratory environment. Currently, we developed several real-time PCR methods, such as single-tube real-time PCR and single-tube nested real-time PCR (Figure 4) to simultaneously detect multiple pathogens in a closed system which has substantially reduced the possibility of false-positive results due to amplification product carryover contamination [24, 25]. Figure 3. A typical result of graphical view from our laboratory. 62 Polymerase Chain Reaction for Biomedical Applications Figure 4. Single closed-tube nested real-time-PCR system: in order to reduce the chance of carryover contamination, all reactions including reverse transcription, conventional PCR, first PCR, nested PCR, and real-time TaqMan detection are performed in a single closed tube [24].

4. Method to detect contamination In the context of this discussion, contamination is defined as the unwanted presence of a PCR amplicon. At times, PCR contamination is present but difficult to ascertain. If a significant contamination problem appears in a PCR laboratory, we need to walk through the procedure of testing for contamination and, if necessary, replace all

reagents. The PCR parameters considered for potential sources of contamination include amplification setup, amplification product handling, and DNA aerosol and storage. Carryover contamination is determined by the following methods in our laboratory. 4.1. Internal controls Appropriate control reactions are helpful in determining whether DNA contamination has occurred. It is important to use a special PCR-positive control which is different from the sample DNA, such as a DNA fragment with a deletion or base alteration in the region of amplification [26]. PCR products can be assessed on a gel to distinguish the control from the native PCR products. Negative controls are also very important and must be included with each run because the first sign of contamination trouble is usually the appearance of an amplification product in the negative or blank controls [27]. 4.2. DNA sequencing Techniques to sequence PCR products were developed in our laboratory in the past few years [15, 28]. This confirmatory sequencing ensures that the PCR product has the expected sequence. The direct comparison of PCR product sequences from a sample and a control is the Regulatory Concern of Polymerase Chain Reaction (PCR) Carryover Contamination
http://dx.doi.org/10.5772/66294 63 best way to determine whether two PCR products are similar or differentȝ After comparison of the DNA sequence variation between the PCR products and the control, the cross contamination of samples can be detected. In some suspected cases, we

directly sequenced PCR products by using the ABI BigDye Terminator Cycle Sequencing kit with a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Brieflyǰ each cycle consists of denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 4 min. After 25 cycles, the fluorescent extension products are purified by a simple isopropanol precipitation step. Software is available on websites [29] to perform a wide range of different types of sequence alignment. DNA sequence data were analyzed by the Geneious, the GenBank sequence database, and the BLAST program from the National Center for Biotechnology Information (NCBI). Accurate identification of any contamination is required for the proper function of FDA field laboratory. The DNA sequencing method should be an ideal technology for this purpose.

5. Discussion PCR is a very powerful**??** and extremely sensitive amplification technique, but there is always the peril that a tiny amount of contamination of the DNA target may lead to false-positive results. It has become necessary to systematically address the issue of PCR contamination, especially in the FDA, a regulatory agency. To overcome this issue, effective methods have been successfully developed and used to avoid carryover contamination in our regulatory laboratories in the past few years [15, 24, 25]. • We have effectively established

and maintained a unidirectional workflow from a PCR clean to a PCR dirty area, thereby reducing the opportunity of contamination to occur. • Samples were set up on a bench that was isolated from PCR product testing areas. • **All PCR master mixes were prepared in a separate room or at least on a separate bench.** Also, we always used a separate laboratory coat, gloves, tubes, and filter pipette tips in the different PCR working areas. • A separate aliquot of water stock for each round of PCR was addressed. • All PCR work benches were decontaminated with 10–15 % bleach and 70 % alcohol. All the pipettesǰ pipette tips, tubes, racks, and gloves were UV-irradiated. • A different pipette tip was used when pipetting each of the PCR reagents, even the same master mix to each tube. • The PCR tubes were kept closed during the procedure. The tubes were opened only when necessary because of potential aerosols that are dangerous with respect to contamination. Minimizing the number of pipetting and mixing steps in PCR master mix preparation is also very important from the perspective of aerosol contamination. • It is very important to schedule PCR when not handling plasmids to prevent cross contamination. 64 Polymerase Chain Reaction for Biomedical Applications • dUTP was incorporated into all PCR products which can subsequently be selectively destroyed by UNG. • Optimization of PCRs is also important. G + C-rich products may be more difficult to inactivate by UNG because of the lower concentration of uridine

triphosphate(UTP). • Positive controls consisted of a low copy number of the desired nucleic acid target and should never be prepared or stored with the samples. • Other amplification methods, such as real-time PCR [23] or closed-tube PCR [25] which can reduce the chances of carryover contamination, are now being used more routinely in our laboratory. • A rapid DNA sequencing method to precisely detect contamination was established. 6. Conclusion Standard precautions should always be employed during all PCR-based testing, whether it is real-time PCR or conventional PCR. All the regulatory laboratories should have their own appropriate controls and systematic measures to prevent and detect contamination. When contamination does occur, we need to accurately determine which reagent is contaminated. All of us should also understand that our individual working habits directly affect our work quality. We believe all the above methods can reduce the risk of contamination and ensure the efficacy of all PCR results. Acknowledgements No official support or endorsement of this article by the Food and Drug Administration is intended or should be inferred. Author details Yuan Hu Address all correspondence to: yuan.hu@fda.hhs.gov U.S. Food and Drug Administration, Northeast Regional Laboratory, Microbiological Sciences Branch, Jamaica, NY, USA Regulatory Concern of Polymerase Chain Reaction (PCR) Carryover Contamination http://dx.doi.org/10.5772/66294 65 References [1] Marx

V. PCR: the price of infidelityȝ Nature Methods. U.S. Food & Drug Administration. 2016;13:475–479. DOI: 10.1038/nmeth.3868 [2] FDA. Bacteriological Analytical Manual (BAM). 2016. [3] Hu Y. Molecular techniques for blood and blood product screening. In: Tang YW, Stratton CW, editors. Advanced Techniques in Diagnostic Microbiology, 2nd ed., Springer, New York, 2015; pp. 513–533. DOI: 10.1007/978-1-4614-3970-7. 28ch [4] Scherczinger CA, Ladd C, Bourke MT, Adamowicz MS, Johannes PM, Scherczinger R, Beesley T, Lee HC. A systematic analysis of PCR contamination. Journal of Forensic Sciences. 1999;44:1042–1045 [5] Czurda S, Smelik S, Preuner-Stix S, Nogueira F, Lion T. Occurrence of fungal DNA contamination in PCR reagents: approaches to control and decontamination. Journal of Clinical Microbiology. 2016;54:148–152 [6] Varanat M, Maggi RG, Linder KE, Horton S, Breitschwerdt EB. Cross-contamination in the molecular detection of Bartonella from paraffin embedded tissues. Veterinary Pathology. 2009;46:940–944 [7] Aslanzadeh J. Preventing PCR amplification carryover contamination in a clinical laboratory. Annals of Clinical & Laboratory Science. 2004;34:389– 396 [8] Mitchell C, Kraft K, Peterson D, Frenkel L. Cross-contamination during processing of dried blood spots used for rapid diagnosis of HIV-1 infection of infants is rare and avoidable. Journal of Virological Methods. 2010;163:489 [9] Tetzner R, Dietrich D, Distler J. Control of carry-over

contamination for PCR-based DNA methylation quantification using bisulfite treated DNA. Nucleic Acids Research. 2007;35 [10] Rouzic E. Contamination pipettingDZ relative efficiency of filter tips compared to Microman® positive displacement pipetteʒ Nature Methods. 2006. DOI: 10.1038/ nmeth887. [11] Ausubel FM. The polymerase chain reaction. In: Ausubel F, Brent R, Moore D, Seidman JG, Smith J, Struhl K, editors. Current Protocols in Molecular Biology, John Wiley & Sons, Inc., U.S.A., Vol. 2, Chapter 15, 1999; 15.01 [12] Cimino GD, Metchette KC, Tessman GW, Hearst JE, Isaacs ST. Post-PCR sterilization: a method to control carryover contamination for the polymerase chain reaction. Nucleic Acids Research. 1990;19(1):99–107 66 Polymerase Chain Reaction for Biomedical Applications [13] Persing DH. PCR Protocols for Emerging Infectious Diseases, ASM Press, Washington, DC, 1996; pp 33–57 [14] Tang Y, Persin DH. Molecular detection and identification of microorganisms. In: Manual of Clinical Microbiology, 7th ed., ASM Press, Washington, DC, 1999; pp 215– 244 [15] Hu Y, Shahidi A, Park S, Guilfoyle D, Hirshfield I. Detection of extrahepatic HCV replication by a novel highly sensitive single tube nested-PCR. American Journal of Clinical Pathology. 2003;119:95–100. [16] Seitz V, Schaper S, Dröge A, Lenze D, Hummel M, Hennig S. A new method to prevent carry-over contaminations in two-step PCR NGS library preparations. Nucleic Acids Research.

2015;10:1093 [17] Sen K. Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples. Publication as an EPA document. U.S.A. 2004 [18] Sefers S, Tang Y. Amplication product inactivation. In: Advanced Techniques in Diagnostic Microbiology, Springer Press, New York, NY, 2006; pp. 306–319 [19] Sambrook J, Russell DW. Molecular Cloning, A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001; 2, ch. 8.17 [20] Atallah Z, Bae J, Jansky S, Rouse D, Stevenson W. Multiplex real-time quantitative PCR to detect and quantify Verticillium dahliae colonization in potato lines that differ in response to Verticillium wilt. Journal of Phytopathology. 2007;97:865–872, [21] Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carryover contamination in polymerase chain reactions. Gene. 1990;93:125–128 [22] Choi YJ, Hu Y, Mahmood A. Clinical significance of a polymerase chain reaction assay for the detection of Mycobacterium tuberculosis. American Journal of Clinical Pathology. 1996;105:200–204 [23] Blackstone GM, Nordstrom JL, Vickery MCL, Bowen MD, Meyer RF, DePaola A. Detection of pathogenic Vibrio parahaemolyticus in oyster enrichments by real time PCR. Journal of Microbiological Methods. 2003;539:149–155 [24] Hu Y, Arsov I. A rapid single-tube protocol for HAV detection by nested real-time PCR. Food and Environmental Virology.

2014;6:189–195 [25] Hu Y, Arsov I. Nested real-time PCR for hepatitis A detection. Letters in Applied Microbiology. 2009:615–619 [26] Goswami B. FDA Bacteriological Analytical Manual Online, Chapter 26 Regulatory Concern of Polymerase Chain Reaction (PCR) Carryover Contamination http://dx.doi.org/10.5772/66294 67 [27] Bacich DJ, Sobek KM, Cummings JL, Atwood AA, O'Keefe DS. False negative results from using common PCR reagents. BMC Research Notes. 2011;4:457 [28] Hu Y, Hirshfield I. Rapid approach to identify an unrecognized viral agent. Journal of Virological Methods. 2005;127:80–86 [29] NIH BLAST. Global Alignment. U.S. National Library of Medicine [Accessed: 2016-07-22] 68 Polymerase Chain Reaction for Biomedical Applications https://cdn.intechopen.com/pdfs/52932.pdf

After Repeated Failures, It's Time To Permanently Dump Epidemic Models

https://issuesinsights.com/2020/04/18/after-repeated-failures-its-time-to-permanently-dump-epidemic-models/
https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/cases-in-us.html
https://www.nejm.org/doi/full/10.1056/NEJMe2002387
https://reason.com/2020/03/30/as-trump-imagines-2-2-million-deaths-from-covid-19-in-the-u-s-a-top-federal-

disease-expert-cautions-against-believing-worst-case-scenarios/

SARS 2003: **fraud**, and the **credibility** of the World Health Organization Apr 20 , 2020 by Jon Rappoport.

History matters. If the World Health Organization (WHO) deceived the world into fear and panic **then**, in 2003, why should you believe them **now** re COVID, when both instances involve epidemics? As some readers will recall, in 2003 the World Health Organization (WHO) put out a **travel advisory**—don't go to Toronto. Toronto was "infected" with epidemic SARS. The loss of tourist income was significant. At the time, I was in touch with a Canadian activist who was trying to assemble a group of Toronto merchants and file a law suit against WHO for a few billion dollars, but it fell apart. The Canadian Encyclopedia describes the wild scene in the country: "The outbreak led to the **quarantine** of thousands…and took an economic toll on Toronto. It also **exposed** the country's ill-prepared health-care system…In late April 2003, the World Health Organization (WHO) issued an **advisory against all non-essential travel** to Toronto. Government officials and experts criticized the decision as being unnecessary…During the "outbreak", thousands of Canadians were **quarantined**. Many **voluntarily** quarantined themselves in their homes. Airports in Toronto and Vancouver **screened** travellers **for high**

fever. **News** coverage spiked with each wave of the outbreak in Toronto and right after the WHO travel advisory. Major Canadian newspapers would each publish up to **25 stories per day** on SARS…" You can see how the World Health Organization stimulated a panicked response with its travel advisory. So SARS must have been a large outbreak, an epidemic of major proportions. Canadian Encyclopedia: "In total, there were 438 probable cases of SARS in Canada, resulting in 44 deaths." **What**?? What about the total number of SARS cases and deaths, worldwide? WHO states: "An epidemic of SARS affected **26 countries**…Other countries/areas in which chains of human-to-human transmission occurred after early importation of cases were Toronto in Canada, Hong Kong Special Administrative Region of China, Chinese Taipei, Singapore, and Hanoi in Viet Nam." Sounds quite serious. The CDC: "During November 2002 through July 2003, a total of 8,098 people **worldwide** became sick with severe acute respiratory syndrome [SARS] that was accompanied by either pneumonia or respiratory distress syndrome (probable cases), according to the World Health Organization (WHO). Of these, 774 died. By late July 2003, **no** new cases were being reported, and WHO **declared the global outbreak to be over."** I see. Across the entire planet, in this sweeping epidemic—8098 cases and 774 deaths. Out of 6.3 billion people.

CDC: “In the United States, only eight persons were laboratory-confirmed as SARS cases. There were no SARS-**related** deaths in the United States.” The capper? Let’s go back to Canada. As the Canadian Encyclopedia states, a mere week or so after WHO declared the “epidemic” was **over**, “English rock band The Rolling Stones headlined a benefit concert in Toronto in response to the outbreak’s economic toll on the city. Informally called ‘SARSStock’ and ‘SARS-a-palooza,’ the concert took place on 30 July 2003. Estimated attendance at Downsview Park was 450,000 to 500,000 people.” Right. And the residue of this “**deadly** virus”—with half a million people standing cheek to jowl—did… Nothing. **History** matters. If you want to believe anything the World Health Organization is claiming now, in 2020, do so at your own peril.

COVID **Italy update:** dispelling the pandemic illusion
Apr 9 , 2020 by Jon Rappoport

The following information on Italy is as of March 30. It comes from an article posted at Swiss Propaganda Research. It describes reports from the Italian National Health Institute. It confirms what I’ve been writing about Italy—which is: Take a population of many elderly people who already have serious, multiple, long-term health conditions, including lung conditions. Note that these people have already been treated with a number of

toxic medical drugs. Add in very toxic air pollution in certain sectors of the country—which, in fact, accounts for a great amount of these lung problems. Consider that **pneumonia**—said to be a cardinal feature of COVID-19—has been **rampant** in Italy for a long time, long before the emergence of the supposed coronavirus. Numbers of flu-like illness cases and pneumonia cases, going back before "the pandemic," are **huge**. These cases show the same general symptoms attributed to COVID. Finally, use a diagnostic test, which, as I've described, can rack up false-positives for reasons that have nothing to do with COVID…and you have the illusion of a new epidemic. "But…but what about the **overflowing ICU wards in hospitals?"** Think it through. Every elderly ill person with lung problems now **fears** he/she might "have the virus," and so comes the flood of people to hospital. It's no mystery. All right. Here are excerpts from the Swiss Propaganda Research article, "Facts about Covid-19": "According to the latest data of the Italian National Health Institute ISS, the **average** age of the positively-tested deceased in Italy is currently about **81** years. 10% of the deceased are over **90** years old. 90% of the deceased are over 70 years old." "80% of the deceased had suffered from two or more chronic diseases. 50% of the deceased had suffered from three or more chronic diseases. The chronic diseases include in particular cardiovascular problems, diabetes, respiratory problems and cancer." **"Less than 1% of the deceased were**

healthy persons, i.e. persons **without** pre-existing chronic diseases. Only about 30% of the deceased are **women**." "The Italian Institute of Health moreover distinguishes between those who died **from** the coronavirus and those who died **with** the coronavirus. In many cases it is **not yet clear** whether the persons died from the virus or from their pre-existing chronic diseases or from a combination of both." "The two Italians deceased [!!] under 40 years of age (both 39 years old) were a cancer patient and a diabetes patient with additional complications. In these cases, too, the exact cause of death was **not** yet clear (i.e. if from the virus or from their pre-existing diseases)." "The partial overloading of the hospitals is due to the general rush of patients and the increased number of patients requiring special or intensive care. In particular, the aim is to stabilize respiratory function and, in severe cases, to provide [toxic] anti-viral therapies." "Northern Italy has one of the **oldest** populations and the **worst** air quality in Europe, which had already led to an increased number of respiratory diseases and deaths in the past and is likely an additional risk factor in the current epidemic." "South **Korea**, for instance, has experienced a much milder course than Italy and has already passed the peak of the epidemic. In South Korea, only about 70 deaths with a positive test result have been reported so far. As in Italy, those affected were mostly **high**-risk patients." "The few dozen test-positive Swiss deaths so far were also high-risk

patients with chronic diseases, an average age of more than 80 years and a maximum age of 97 years, whose exact cause of death, i.e. from the virus or from their pre-existing diseases, is **not** yet known." I'll clarify a further point. Even if the diagnostic tests on patients claim to show the presence of the COVID-19 virus—and even if we accept that finding as true—the test has **never** been proved to be able to say **HOW MUCH** virus is in a patient's body. And that is vital, because, to even begin talking about a person actually getting sick, he would have to have millions **and** millions of virus actively replicating in his body. Therefore, **the finding of the test is irrelevant in the real world, as opposed to the lab.** In the real world, of which Italy is a part, people who are aged, who have multiple and very serious long-term health problems, who have been treated for years with **toxic** drugs—these people die of those factors. There is **no** need for a purportedly new virus to explain why they are dying. The absolute fraud and crime involved here are enormous. The perpetrators, **in their bubble of reputation, wall-to-wall false science, media robots, and government back-up** will escape with their careers intact. But the truth has a way of toppling pedestals and the people who stand on them.

Corona: **creating the illusion of a pandemic through diagnostic tests** Apr 8 by Jon Rappoport

Nailed them, with their own words. In this article, I'll present quotes from official sources about their own diagnostic test for the coronavirus. I'm talking about fatal **flaws** in the test. Because case numbers are based on those tests **(or no tests at all), the whole "pandemic effect" has been created out of fake science.** In a moment of truth, a propaganda pro might murmur to a colleague, "You know, we've got a great diagnostic test for the virus. The test turns out all sorts of results that say this person is diseased and that person is diseased. Millions of diseased people. **But the test doesn't really** measure that. The test is **ridiculous**, but ridiculous in our favor. **It builds the picture of a global pandemic.** An **excuse** to lock down the planet and wreck economies and lives…" The widespread test for the COVID-19 virus is called the PCR. I have written much about it in past articles. Now let's go to **published official literature,** and see what it reveals. Spoiler alert: the **admitted holes** and shortcomings of the test are **devastating**.

From "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" [1]: "Detection of viral RNA may **not** indicate the presence of infectious virus or that 2019-nCoV is the **causative** agent for clinical symptoms." **Translation**: A positive test doesn't guarantee that the COVID virus is causing infection at all. And, ahem, reading between the lines, maybe the COVID virus might **not** be in the patient's body at all, either.

From the World Health Organization (WHO): "Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans" [2]: "Several assays that detect the 2019-nCoV have been and are currently **under** development, both in-house and commercially. Some assays may detect only the novel virus [COVID] and some may also detect other strains (e.g. SARS-CoV) that are genetically **similar**." **Translation**: Some PCR tests register positive for types of coronavirus that have nothing to do with COVID—including plain old coronas that cause nothing more than a cold. The WHO document **adds** this little piece: "Protocol use **limitations**: Optional clinical specimens for testing has [have] **not yet been validated."** Translation: We're **not** sure which tissue samples to take from the patient, in order for the test to have any validity.

From the FDA: "LabCorp **COVID-19RT-PCR test EUA Summary: ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARYCOVID-19 RT-PCR TEST (LABORATORY CORPORATION OF AMERICA)" [3]: "…The SARS-CoV-2RNA [COVID** virus] is generally detectable in respiratory specimens during the **acute** phase of infection. Positive results are **indicative** of the presence of SARS-CoV-2 RNA; **clinical correlation** with patient history and **other diagnostic** information is necessary to determine patient infection status…**THE AGENT DETECTED MAY NOT BE THE DEFINITE CAUSE OF DISEASE**.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health **authorities**." Translation: On the one hand, we **claim** the test can "generally" detect the presence of the COVID virus in a patient. **But we admit** that "the agent detected" on the test, by which we mean COVID, "may **not** be the definite cause of disease." **We also admit that,** unless the patient has an **acute** infection, we **can't** find COVID. Therefore, **the idea of "asymptomatic patients" confirmed by the test is nonsense.** And even though a positive test for COVID may **not** indicate the **actual** cause of disease, all positive tests **must** be reported—and they **will** be counted as "COVID cases." Regardless.

From a manufacturer of PCR test kit elements, Creative Diagnostics, "SARS-CoV-2 Coronavirus Multiplex RT-qPCR Kit" [4]: "Regulatory status: **For research use only, not for use in diagnostic procedures."** Translation: Don't use the test result **alone** to diagnose infection or disease. Oops. "non-specific interference of Influenza A Virus (H1N1), Influenza B Virus (Yamagata), Respiratory Syncytial Virus (type B), Respiratory Adenovirus (type 3, type 7), Parainfluenza Virus (type 2), Mycoplasma Pneumoniae, Chlamydia Pneumoniae, etc." **Translation**: Although this company states the test can detect COVID, it **also states** the test can read FALSELY positive **if** the patient has one of a

number of other irrelevant viruses in his body. **What is the test proving, then? Who knows? Flip a coin.** "Application Qualitative" **Translation**: This clearly means the test is **not** suited to detect how much virus is in the patient's body. I'll cover how important this admission is in a minute. **"The detection result of this product is only for clinical reference, and it should not be used as the only evidence for clinical diagnosis and treatment.** The clinical management of patients should be considered in combination with their symptoms/signs, history, **other** laboratory tests and treatment responses. The detection results should **not** be directly used as the evidence for clinical diagnosis, and **are only for the reference of clinicians."** Translation: **Don't** use the test as the exclusive basis for diagnosing a person with COVID. And **yet**, this is exactly what health authorities are doing all over the world. All positive tests must be reported to **government** agencies, and they are counted as COVID cases."

Those quotes, from **official** government and testing sources, **torpedo** the whole "scientific" basis of the test. And now, I'll add another, lethal blow: **the test has never been validated properly as an instrument to detect disease.** Even **assuming** it can detect the presence of the COVID virus in a patient, it **doesn't** show **how much** virus is in the body. And that is key, because in order to even begin talking about actual illness in the **real** world,

not in a lab, the patient would need to have millions and millions of the virus actively replicating in his body. Proponents of the test assert that it can measure **how much** virus is in the body. To which I reply: **prove it.**

The test is an unproven fraud. And, therefore, the COVID **pandemic, which is supposed to be based on that test, is also a fraud.** "But…but…what about all the sick and dying people…why are they sick?" I've written thousands of words answering that question, in past articles. A number of conditions—none involving COVID, and most involving old traditional diseases—are making people sick.

SARS-CoV-2 Coronavirus Multiplex RT-qPCR Kit (CD019RT) **Regulatory status: For research use only, not for use in diagnostic procedures.**
https://www.creative-diagnostics.com/sars-cov-2-coronavirus-multiplex-rt-qpcr-kit-277854-457.htm

LabCorp **COVID-19 RT-PCR test EUA Summary 1**
ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY COVID-19 RT-PCR TEST (LABORATORY CORPORATION OF AMERICA)
For In vitro Diagnostic Use Rx
Only For use under Emergency Use Authorization (EUA) only https://www.fda.gov/media/136151/download
Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance
CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel For Emergency Use Only Instructions for Use
https://www.fda.gov/media/134922/download

COVID: **here come the antibody tests—quick, easy, and insane** Apr 5 , 2020 by Jon Rappoport

There are **two** worlds. In the **first**, independent researchers with no conflicts of interest, and, hopefully, a sense of logic, sort out what is actually going on behind **propaganda parading as medical research.** In the **second** world, it's all official propaganda, wall to wall, posing as science. This article looks at the second world. It doesn't mention what I've established in prior articles (full archive https://blog.nomorefakenews.com/category/covid/): the **unproven** discovery of a new virus (COVID); the **notoriously useless** PCR diagnostic test for the virus, rendering case numbers **meaningless**; the **con-job** proposition that COVID is a real disease with one cause, rather than a **grouping** of people with diverse conditions **clustered under one fake umbrella term** (COVID). In the second world, we have the announcement that **a new antibody test has been** developed to detect COVID-19 virus in people. Millions of **test kits** have been ordered.

Some versions of the test can be **self**-administered quickly at home. So let's go to the mainstream media and see what they, and their medical sources, have to say about the new **antibody** test. Buckle up. **Chicago Tribune,** April 3: "A new, different type of coronavirus **test** is coming that will help significantly in the fight to quell the COVID-19 pandemic, doctors and scientists say." "The **first so-called** serology test, which detects **antibodies** to the virus rather than the virus itself, was given emergency approval Thursday by the U.S. Food and Drug Administration." "The serology test involves taking a blood sample and determining if it contains the antibodies that fight the virus. A positive result indicates the person had the virus in the past **and** is currently immune."

"Dr. Elizabeth McNally, director of the Northwestern University Feinberg School of Medicine Center for Genetic Medicine…'You'll see many of these roll out in the next couple of weeks, and it's great, and it will really help a lot,' said McNally, noting doctors and scientists will be able to use it to determine just how widespread the disease is, who can safely return to work and possibly how to develop new treatments for those who are ill." Got that? **A positive test means the patient is now immune to the virus and can walk outside and go back to work.**

NBC News, April 4, has a somewhat **different** take: "David Kroll, a professor of pharmacology at the

University of Colorado who has worked on antibody testing, explained that the antibodies [a positive test] mean 'your immune system [has] remembered the virus to the point that it makes these antibodies that could inactivate **any future** viral infections'." "What the test **can't** do is tell you whether you're **currently** sick with coronavirus, whether you're contagious, whether you're fully immune — and whether you're safe to go back out in public.""Because the test **can't** be used as a **diagnostic** test, it would need to be combined with other information to determine if a person is sick with COVID-19." Oops. No, this **really isn't a** diagnostic test, it doesn't tell whether the patient is immune and can go back to work. Excuse me, what??

Business Insider, April 3: "The world's leading industrial nations have so far **failed to identify any coronavirus antibody tests that will be accurate enough for home use,** according to the UK's Health Secretary Matt Hancock." "The UK and other nations are currently examining plans to use antibody tests to allow individuals with immunity to COVID-19 to exit their national lockdowns early through the use of a so-called 'immunity passport'." "Spain was recently forced to return tens of thousands of rapid coronavirus tests from a Chinese company after they were found to be **accurate just 30% of** the time." "Some tests have demonstrated false positives, detecting antibodies to much more common

coronaviruses." "Scientists also remain unsure about the extent to which a **past** infection could prevent reinfection and how long an immunity would remain." Hmm. So the **new antibody test has very serious** problems, and it **hasn't** been cleared for public use.

Medicine Net (undated): "Researchers at the Mount Sinai Health System say they've developed a test that can find out if you **already have had or were** infected with the new coronavirus." "The test is called "serological enzyme-linked immunosorbent assay," or ELISA for short. It checks whether or not you have antibodies in your blood to SARS-CoV-2, the scientific name of the new coronavirus that causes COVID-19." "Researchers say ELISA works like antibody tests for **other** viruses, such as hepatitis B. It will show whether your immune system — the body's defense against germs — made contact with SARS-CoV-2, even months **before**." "The test could help scientists fight the pandemic in several ways. It can give researchers a more accurate measure of how many people had the new coronavirus. It would also let health care workers who were ill with COVID-19 symptoms, but were **never** tested for the disease, return to work — confident that they are now immune." So wait, it's a great test. Right? **A positive test result indicates** immunity, and people can return to work. What??

Science News, March 27: “The United Kingdom has ordered 3.5 million antibody tests, which would show whether someone has been exposed to COVID-19. Such tests, which just take a **drop** of blood, could help reveal people who have been **exposed** to the virus and are now likely immune, meaning they could go back to work and resume their normal lives.” “Science News spoke with David Weiner, director of the Vaccine and Immunotherapy Center at the Wistar Institute in Philadelphia, and Charles Cairns, dean of the Drexel University College of Medicine, about **how antibody tests work and what are some of the challenges of developing the tests.”** “Cairns: ‘The big question is: Does a positive response for the antibodies mean that person is actively infected, or that they have been infected in the past? The tests need to be accurate, and avoid both false positives and false negatives. That’s the challenge’.” Oops again. Cairns is saying the **new test, if it reads positive, might mean the person is infected now. Or it** might mean they were infected—and are now presumably immune. **Figuring out which is the challenge.** No kidding. It’s the difference between sick and healthy. So a positive test result means the patient is **sick OR healthy.** As a reference, let’s look at how this **same** antibody test has been used in the **past**. For example, in the case of hepatitis A:

URMC Rochester (undated): This test looks for antibodies in your blood called IgM. The test can find out

whether you are infected with the hepatitis A virus (HAV) …If your test is positive or reactive, it may mean: You have an active HAV infection…You have had an HAV infection within the last 6 months." In other words, a positive antibody test could mean you're sick **now, or were once sick but** are presumably immune now. Wonderful.

Medscape comments on the meaning of a positive antibody test for the Zika virus: "…immunoglobulin (Ig) M and **neutralizing** antibody testing can identify additional recent Zika virus infections…However, Zika virus antibody test results can be **difficult to interpret because** of cross-reactivity with other flaviviruses…" **Two** things here: **no word about a positive test result revealing immunity from Zika; and a warning that a positive test might not have anything to do with Zika at all—that's what "cross-reactivity" means.**

Medlineplus, referring to a Zika "blood test," which would include antibody testing, states, "A positive Zika test result **probably** means you have a Zika infection." **Not** immunity. And there you have it.

The official word on the COVID antibody test from official sources. It's yes, no, and maybe.

Public health officials can say **whatever** they want to about antibody tests: a positive result means you're

immune, it means you have an infection, it means you're walking on the moon eating a hot dog.

Generally speaking, **before** 1984 a positive antibody test was taken to mean the patient had achieved immunity from a germ. **After** 1984, the science was turned upside down; a positive result meant the patient "had the germ" and was not immune. **Now**, with COVID-19, if you just read news headlines, a positive test means the patient is immune; **but if you** read down a few paragraphs, a positive test means the patient is maybe…maybe not… immune. Maybe infected, maybe not infected. Maybe sick, maybe not sick. And, on top of all that, antibody tests are known to read **falsely** positive, owing to factors that have nothing to do with the virus being tested for.

That concludes today's foray into the world of **lunatic contradictory** propaganda masquerading as science. You are now returned to the real world, where: the discovery of a **new** virus (COVID) is **unproven**; the **notoriously useless** PCR diagnostic test for the virus renders case numbers meaningless; and the proposition that COVID is a real disease with one cause is a **con job.**

SOURCES:
https://www.chicagotribune.com/coronavirus/ct-coronavirus-antibody-test-20200403-i6wzmddt5zffpeqgk4xbwmkbmy-story.html

https://www.nbcnews.com/health/health-news/home-fingerprick-blood-test-may-help-detect-your-exposure-coronavirus-n1176086
https://www.businessinsider.com/coronavirus-antibody-test-g7-leaders-accuracy-covid-19-immunity-passports-2020-4
https://www.medicinenet.com/script/main/art.asp?articlekey=229579
https://www.massdevice.com/fda-clears-bodysphere-2-minute-covid-19-test
https://www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf
https://www.sciencenews.org/article/covid-19-coronavirus-pandemic-how-antibody-blood-tests-work
https://www.mayoclinic.org/diseases-conditions/swine-flu/diagnosis-treatment/drc-20378106
https://www.verywellhealth.com/h1n1-swine-flu-diagnosis-4163091
https://www.webmd.com/cold-and-flu/flu-guide/h1n1-flu-virus-swine-flu#2-4
https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=hepatitis_a_antibody
https://www.medscape.com/viewarticle/864228
https://medlineplus.gov/lab-tests/zika-virus-test/
http://www.immunity.org.uk/articles/christine-johnson/

The Creation of a False Epidemic with Jon Rappoport – Parts I – III https://home.solari.com/the-creation-of-a-false-epidemic-with-jon-rappoport/

https://www.youtube.com/watch?v=zbjQ1sC0ahk

There are people in Wuhan who have pneumonia because of the horrendous air quality in the city. There are people in New York who have ordinary flu-like illness. There are people in Italy who have histories of multiple, long-term, serious health conditions—pneumonia, flu, cardiac problems, kidney problems—made far worse through treatment with toxic drugs. There are people in hospitals around the world who, after being diagnosed with COVID, are dosed with powerful **toxic** antiviral drugs. There are people on breathing ventilators who are being given **too much** oxygen and **too much** pressure—and their lungs collapse. There are perfectly healthy people who are testing positive for the virus because the test is **irrepairably flawed**… All these people are called "COVID cases."

More **non**-virus causal factors in "epidemic cases"—hospitals by Jon Rappoport March 31, 2020
As my long-time readers know, since 1987 in my investigations of **fake** epidemics, I've deployed the strategy of finding **actual** causes of illness and death that have nothing to do with the latest and greatest **hype** about

a “new virus” creating widespread harm. In other words, I show there is **no** need to invoke a novel and unproven virus, in order to explain the so-called epidemic effects. I have been doing that all along during this **false** COVID pandemic. In today’s episode of medical worshipers go crazy and virus fakery, let’s go to the hospitals to find yet more **NON**-VIRUS causes of illness and death in supposed “coronavirus patients.” **Three** questions: If hospitals are overwhelmed with patients, as night follows day it must be the coronavirus. Right? **WRONG.** If patients are on breathing ventilators, as night follows day their problem must be the coronavirus. Right? **WRONG.** If patients are being put on **ibuprofen**, as night follows day their problem must be the coronavirus. Right? **WRONG.**
Before I explain what “wrong” means in each instance, an overview of hospital care in the US is instructive. The **reference** is Journal of the American Medical Association, July 26, **2000**, Dr. Barbara Starfield, a revered public health expert at the Johns Hopkins School of Public Health. Starfield’s review was: **“Is US Health Really the Best in the World?”** She blows the whistle on her own elite colleagues and vast numbers of other medical providers. Among her findings: Annual number of deaths caused by mistreatment and errors in US hospitals: 119,000. This should give pause for thought. **Instead** of blithely **assuming** that so-called coronavirus patients who die in hospitals are dying from the virus, consider the **effects** of care **IN** the hospitals. Now let’s get

to the **three** questions I asked above. What about overwhelmed hospitals? Surely, this must mean coronavirus cases are the cause, right? **What else** could it be? Overwhelmed hospitals are a **new** phenomenon, paralleling the rise of COVID, right? Here, from Time magazine, is a sample report from **2018**, **long BEFORE COVID supposedly emerged.** "Hospitals overwhelmed by **flu** patients are treating them in **tents**": "The 2017-2018 **influenza** epidemic is sending people to hospitals and urgent-care centers in every state, and medical centers are responding with extraordinary measures: asking staff to work overtime, setting up triage **tents**, **restricting** friends and family visits and canceling elective surgeries, to name a few." "'We are pretty much at capacity, and the volume is certainly different from **previous flu** seasons'," says Dr. Alfred Tallia, professor and chair of family medicine at the Robert Wood Johnson Medical Center in New Brunswick, New Jersey. 'I've been in practice for 30 years, and it's been a good 15 or 20 years **since** I've seen a flu-**related** illness scenario like we've had this year'." "Tallia says his hospital is 'managing, but just barely,' at keeping up with the increased number of sick patients in the last three weeks. The hospital's **urgent**-care centers have also been inundated, and its outpatient clinics have no appointments available." "The story is **similar** in Alabama, which declared a state of **emergency** last week in response to the flu epidemic. Dr. Bernard Camins, associate professor of infectious diseases at the University

of Alabama at Birmingham, says that UAB Hospital cancelled elective surgeries scheduled for Thursday and Friday of last week to make more beds available to flu patients." "'We had to treat patients in places where we normally wouldn't, like in recovery rooms,' says Camins. 'The **emergency** room was very crowded, both with sick patients who needed to be admitted and patients who just needed to be seen and given [**toxic**] Tamiflu'." "In California, which has been particularly **hard** hit by this season's flu, several hospitals have set up **large 'surge tents'** outside their emergency departments to accommodate and treat flu patients. Even then, the LA Times reported this week, emergency departments had standing-room only, and some patients had to be treated in hallways." "The Lehigh Valley Health System in Allentown, Pennsylvania, set up a similar surge tent in its parking lot on Monday, in response to an increase in patients presenting with **various** viral illnesses, including norovirus, respiratory syncytial virus (RSV) and the flu. 'We've put it into operation a couples times now over the last few days,' said a hospital spokesperson. 'I think Tuesday we saw upwards of about 40 people in the tent itself'." "Many hospitals are also encouraging visitors to stay away. Kaiser Permanente Los Angeles Medical Center announced last week that it was temporarily restricting visits from **children** 14 and under and anyone with flu symptoms. 'This measure is to prevent unnecessary spread of influenza and to protect you, our

patients, and our staff,' the health system posted on Facebook." "Loyola University Health System in Chicago —which set a hospital flu-activity record of 190 confirmed cases between January 7 and 13—has also instituted similar visitor restrictions, although a spokesperson for the hospital says it's a standard precaution for flu season. Loyola also requires all employees to receive a mandatory flu shot, a policy it started in 2009." "In Fenton, Missouri, SSM Health St. Clare Hospital has opened its emergency overflow wing, as well as all outpatient centers and surgical holding centers, to make more beds available to patients who need them. Nurses are being 'pulled from all floors to care for them,' says registered nurse Jennifer Braciszewski, and are being offered an increased hourly rate to work above and beyond their normal schedules. Many nurses have also become **sick**, however, so the staff is also short-handed…" —All this, **before** 2019. **Before the "epidemic."** You can find other stories of such hospital problems. In **Italy**, for example, before the "epidemic," the waiting lists for hospital appointments could stretch out for **months - revealing the whole system was heavily stressed, already overburdened, and** short-staffed **before** the latter part of 2019. **Second** question: If patients are on breathing ventilators, as night follows day their problem must be the coronavirus. Right? Not necessarily. For example, what about **potential adverse effects of the ventilators themselves?** From the US

National Institutes of Health, here is a **list of those effects.** As you read them, keep in mind that many hospital patients entering the wards **already** have pneumonia (and, of course, **breathing** problems): **"One of the most serious and common risks of being on a ventilator is pneumonia.** The breathing tube that's put in your airway can allow bacteria to enter your lungs. As a result, you may develop ventilator-**associated** pneumonia (VAP)." "The breathing tube also makes **it hard for you to cough.** Coughing helps **clear** your airways of lung irritants that can cause infections." "VAP is a major concern for people using ventilators because they're often **already very sick.** Pneumonia may make it **harder to treat their other disease or condition** [like **PNEUMONIA**]." "…Using a ventilator **also** can put you at risk for **other** problems, such as: * Pneumothorax. This is a condition in which **air leaks out of the lungs and into the space between the lungs and the chest wall.** This can cause pain and shortness of breath, and it may cause one or both lungs to **collapse**. * Lung **damage**. Pushing air into the lungs with too much pressure can harm the lungs. * Oxygen/air **toxicity**. High levels of oxygen can damage the lungs." "These problems may occur because of the **forced** airflow or high levels of oxygen from the ventilator." "Using a ventilator also can put you at risk for blood **clots** and serious skin infections. These problems tend to occur in people who have certain diseases and/or who are confined to bed or a wheelchair and must remain

in one position for long periods…" **Third** question: can **ibuprofen** cause problems? From drugs.com, here is a **list** of adverse effects from Advil: "Advil can increase your risk of **fatal** heart attack or stroke, especially if you use it long term or take high doses, or if you have heart disease. Even people **without** heart disease or risk factors could have a stroke or heart attack while taking this medicine." "Do not use this medicine just before or after heart bypass surgery (coronary artery bypass graft, or CABG)." "Advil may also cause stomach or intestinal bleeding, which can be **fatal**. These conditions can occur **without** warning while you are using ibuprofen, especially in **older** adults." "You should not use Advil if you are allergic to ibuprofen, or if you have ever had an **asthma** attack [breathing problems] or severe allergic reaction **after** taking aspirin or an NSAID." "Ask a doctor or pharmacist if it is safe for you to take this medicine if you have: * heart disease, high blood pressure, high cholesterol, diabetes, or if you smoke; * a history of heart attack, stroke, or blood clot; * a history of stomach ulcers or bleeding; * asthma; * liver or kidney disease; * fluid retention; or * a connective tissue disease such as Marfan syndrome, Sjogren's syndrome, or lupus." "Taking Advil during the last 3 months of **pregnancy** may harm the unborn baby.Do not use this medicine without a doctor's advice if you are pregnant." "It is **not** known whether ibuprofen passes into breast milk or if it could affect a nursing baby. Ask a doctor before using this medicine if

you are breastfeeding." NOTE: Antiviral drugs, given to many people diagnosed with COVID, have **serious** toxic adverse effects. Getting the picture? It isn't always the reason a person **COMES** to hospital which causes the worst problem. It can be what happens **IN** the hospital, including **death**. Unrelated to any purported COVID virus. And yet, the increased illness or death would be written up as a "coronavirus case."

People dying equals Coronavirus? An engineered virus?
by Jon Rappoport March 30, 2020
This article is intended for **close** study. I urge you **not** to jump to an early conclusion about what I'm proposing here. For example, I'm not ruling out the engineering of a virus. But an unusual twist is involved. This article is **also part of a larger** position.
That position can be defined as: **AUTOMATICALLY ASSUMING THE SO-CALLED EPIDEMIC IS CAUSED BY A VIRUS IS INCORRECT. THE TRUTH DOESN'T SUPPORT FEAR. FEAR IS SELF-DEFEATING. FEAR CLOSES DOWN POSSIBILITY. DON'T CLOSE DOWN POSSIBILITY IN ANY ASPECT OF YOUR LIFE.**
Among other subjects, this article comments on the **hypothesis** that the COVID-19 virus is a **modified weaponized germ from a lab**, either deliberately or accidentally released. A general comment: weaponizing a virus as an instrument for causing widespread destruction faces a significant **barrier**. From the get-go, viruses mutate very quickly as they replicate. Therefore, the

criminals **wouldn't** maintain the viral structure they started with. Ensuring continued lethality would therefore appear to be **impossible**. Then there is this: I fully understand that researchers in certain labs are always fiddling and diddling with viruses. That's their job. The question, in a given situation, is: are they successful at weaponization, even ignoring the rapid mutation factor I just mentioned? **THERE IS A MAJOR DIFFERENCE BETWEEN: THEY "TRIED" TO WEAPONIZE A CORONAVIRUS IN LABS, AND THEY "SUCCEEDED" AND RELEASED IT. EVIDENCE FOR THE FORMER, NO MATTER HOW CONVINCING, IS NOT EVIDENCE OF THE LATTER.**

Anything is possible, but so far, what I see is this: when I add up all the reasons people are sick and dying, I **don't** see a new germ as the basis. I've detailed, in past articles, all the Chinese cases who have been diagnosed for no other reason than they have pneumonia, a **traditional** disease of major proportions in China. Studies estimate that roughly 300,000 citizens **die of it every year.** Which means there are **millions** of Chinese people who develop pneumonia each year. Furthermore, the Chinese **government** quickly **abandoned** the idea of testing for the purported coronavirus - **favoring instead, CT scans of the lungs.** A finding of pneumonia was **sufficient** for a diagnosis of an "epidemic case." That is **absurd** on its face. Pneumonia has **many causes, none of which requires a new virus.** Then we have the cases in **Italy**, the **second** largest reservoir of the so-called epidemic. Here, the deaths occur **massively** on the side of the

elderly, who already have serious prior medical conditions, long term. In the reports issued by the **Italian government** so far, the people dying are said to "have the virus," **but** the conclusion is they're dying because of their prior medical conditions. The conventional wisdom, often spouted, is: "the coronavirus strikes the elderly, who are less able than the young to ward it off." This is a misnomer, **deployed to cover up** the reality that the elderly are passing away, as they usually do, from the illnesses they already have- **no** need for a new virus. I've also discussed deaths in Australia and the state of Washington. Again, it's elderly people. As in Italy, add up their long-term diseases; **the treatment of those diseases with toxic medical drugs; the fear engendered by the diagnosis of** "**COVID**"; **sudden isolation** from family and friends; the **use** of breathing ventilators, which have their own set of adverse effects, including bacterial pneumonia; and new treatment with **toxic** antiviral drugs, to "fight the virus"; and you have a **terribly potent array of factors** which account for the elderly dying. **No** need for a new virus. As I've detailed in past articles, flu-**like** diseases (quite often, with **no** evidence of a flu virus) are **traditional** in Europe and the US. Their symptoms **overlap** the symptoms listed for so-called **COVID**. In **recent** years, there have been **huge** numbers of such people with these flu-**like** illnesses, and many have died —**before** the emergence of the so-called **COVID** virus. Again, no need for a unique new virus. And as far as

overall global case numbers of **COVID** are concerned, a **large** percentage of these people have been diagnosed **purely on the basis of their symptoms,** with no test, or via the accepted diagnostic test, called PCR. I'll cover that test in a moment. Suffice to say, it **fails** to prove illness is stemming from **COVID** virus or any virus—but it does create a picture of supposedly **swelling** case numbers. In a recent article, I've quoted the literature of official public health authorities, who themselves admit the test has fatal flaws. Then we have **unexplained** relatively small clusters of people who appear to be suddenly falling ill. A **closer** examination of these people is necessary, to see whether they, in fact, are "sudden and unexplained." And **rather** than an engineered virus—which has unpredictable effects owing to its rapid mutation—**if** we're looking for sinister operations, I suggest that, **to cause** sensational alarm and bafflement and "proof" that a mysterious event is underway, the **intentional** seeding of locales with little-known toxic chemicals , would be the action undertaken. The effects of **chemicals** are far more predictable in terms of intensity and duration, and **if no one is specifically looking for them, they are undetectable.** Finally, in major cities of China (e.g., Wuhan) and Italy (particularly in the north), highly **toxic** air quality has been far more than "a serious problem" for some years. This **alone** would account for huge numbers of people suffering from **all sorts of lung** conditions, **including** pneumonia. Pneumonia is one of

the **cardinal** listed symptoms of the "epidemic." In China, the **mix** of toxic pollutants in the air is **un**precedented in human history, spanning both early and modern eras of industrialization.

Conclusion: All in all, I would say that, if a weaponized coronavirus has been achieved, and then released or accidentally leaked, it is **not** a success. Far too much of what is being called **COVID** is explained by the causative factors I've just presented. In fact, if we want to talk about engineered viruses—including what would probably be an **easier** technical job in the lab—the most successful operation would involve slightly altering a common coronavirus to cause nothing more than a common cold. **Then**, with a "self-fulfilling prophecy" diagnostic **test** in hand, people all over the world would test positive; many case numbers would thus be created; and with the **non**-virus illness-causes I've just described, the **illusion** of a global pandemic would be stitched together—**all leading to the real goal**: **LOCKDOWNS,** economic destruction, and the further **pacification** of the population. A bereft population **more dependent than ever on governments and official authorities.** A dazed population **guided** into a **heavily technocratic future**—wall to wall surveillance, smart cities, Internet of Things, universal guaranteed income tied to social credit score. **Most importantly:** Assigned energy quotas for every citizen. **CONTROL**. Moving on from biowar labs to ordinary labs, has the **COVID** virus **ever actually been**

DISCOVERED and isolated there , by proper procedures? As I've written in another article, **COVID** -19 **lacks** correct proof in that regard.

The absence of true isolation and discovery permits such a virus to slip in under the radar. The widespread diagnostic test for the **COVID** virus now in use, called the PCR, falls far short of proving that **any** person is sick or will get sick. In other articles, I have proposed a vetting process for the PCR—which should have been done decades ago—in order to show it works or doesn't work in the real world. This vetting procedure would be suggested by any college science student as obvious and necessary. It has **never** been carried out. It involves proving , the test can determine that a huge quantity of virus, actively replicating in the human body, is present—and therefore, the patient would, in the real world, be sick. Carrying out such a test, on hundreds of patients, in a controlled and blinded setting, **AND THEN SEEING WHETHER THE TEST DOES POINT TO ACTUALLY SICK PEOPLE,** has **never** been done. Therefore, **claiming the test confirms that COVID virus is causing great damage is unsupported.** This, too, is quite **convenient**, if a common coronavirus that causes nothing more than a common cold has been **engineered**. In that situation, **you would want a diagnostic test that can't predict or detect serious illness,** because the virus **doesn't** cause serious illness. The **virus is only there as a prop, to create the illusion of case numbers stemming from one source:** a harmless

COVID -19 virus. Now, let's move on to the effects of propaganda. People say: patients are sick and dying all over the world—so **IT MUST BE THE VIRUS. WHAT ELSE COULD IT BE?**

Aristotle worked out the fact that the effect does not prove the cause. The effect (people sick and dying) does not prove the cause (**COVID** virus). And history matters. It offers clues and precedents. We've seen dud epidemics in the past **blamed** on a virus, and yet, embarrassingly, the virus **couldn't** be found. **BUT WHO CARES, PEOPLE SAY,** moved by propaganda. **IT MUST BE THE VIRUS.** (See my articles on **SARS and Swine Flu 2009.**) —People sick, people dying. How many people? **Unknown**. Massive lockdowns of Chinese cities. Citizens trying to escape. For the global **audience**, **this equals coronavirus,** not because they know the virus is the cause—proof is beside the point. The virus is the cause because **IT MUST BE. WHAT ELSE COULD IT BE?** When **brutal air** pollution in Wuhan obviously brings on **lung** disease; and when the **primary** symptom of the coronavirus is supposed to be **lung** disease; and when citizens of the city have been falling ill and dying from **lung** disease **long before the virus appeared—does this matter? OF COURSE NOT. IT MUST BE THE VIRUS.**

Propaganda. When governments and corporations have been using **THE VIRUS** as a cover story to obscure and explain away their **crimes** against populations, for

decades and decades—does this matter? When previous **so-called** epidemics—for example, West Nile, **SARS**, Zika and Swine Flu- turned out to be complete **unproven** duds- does this history matter? **OF COURSE NOT. IT HAS TO BE THE VIRUS. WHAT ELSE COULD IT BE?**

A face on a television screen watched by millions of people says **CORONAVIRUS. Therefore, case closed.** In 2009, in La Gloria, Mexico, on a giant commercial pig farm, pig feces and urine are allowed to bake and steam and bubble in the sun. These deposits are called lagoons. They're **so large,** you can see them from outer space. Toxic chemicals are routinely sprayed and laid out like whipped cream on the lagoons. Workers are falling ill. New workers are brought in to spray even more toxic chemicals. Workers **die**. Then the Centers for Disease Control sends in their tuned-up **virus** hunters to look for the germ causing the "mysterious" illness. They **claim** to find a Swine Flu virus. **IT MUST BE THE VIRUS. WHAT ELSE COULD IT BE? THE DECAYING PIG SHIT AND URINE?** The layers of poisonous chemicals? Don't be ridiculous. Later, in the summer of 2009, CBS investigative reporter Sharyl Attkisson discovers that the overwhelming percentage of tissue samples from US Swine Flu patients are coming back from labs with **no** sign of **ANY KIND OF FLU.** The virus isn't there.

And yet, of course, we have this, written in the summer of 2009: From healthwyze.org: "The U.S. Patent and

Trademark Office has a **patent** for, Genetically Engineered Swine Influenza Virus and Uses Thereof (patent #8124101). It was filed in **2005** for approval. The makers of the human variant of the swine flu virus waited until the patent was finally approved in January of 2009, before unleashing the virus into the wild. The makers of the swine flu **vaccine** had begun the lengthy patenting process **long before** the swine flu supposedly existed, which means that **the outbreak was no accident, and** the virus is clearly not natural. Patents only apply to man-made items, and natural things **cannot** be patented. The virus conveniently went public **only** after its vaccine patent was approved, after patiently waiting 3 years for that to happen. The pandemic was **declared** just five months **after** the patent was approved, in June of 2009. The tremendous **hysteria** following the outbreak was promoted by the **same** groups who had invented this genetically engineered virus. The word 'invented' was actually used to describe the virus in the patent application." What do you know about that? Back then, there were **reports** that the Swine Flu Virus- which **couldn't** even be found in the overwhelmingly number of US patients- was actually a **biowarfare** germ. Sound **familiar**? Swine Flu was a dud. **Another epidemic that was going to infect the world? West Nile Virus. Another dud.** But here from an old **whale.to** article: **"None of these theories** [about West Nile] has deterred Vermont Senator Patrick Leahy from urging federal

officials to determine if the introduction of WN virus is a **terrorist** attack. On September 12, 2002, Leahy declared: 'I think we have to ask ourselves: Is it a coincidence that we're seeing such an **increase** in WN virus – or is that something that's being tested as a **biological** weapon against us'." Sound familiar? And here, from an old article at rediff.com, a piece about another epidemic dud, SARS 2003 (800 people died out of 7 billion, and WHO researcher, **Frank Plummer**, told the press they couldn't even find the virus in all but a few Canadian patients): "The virus of atypical pneumonia, better known as SARS, or Severe Acute Respiratory Syndrome, was **created** artificially, possibly as a bacteriological **weapon**, Sergei Kolesnikov, academician of the Russian Academy of Medical Sciences, told a press conference in the Siberian town of Irkutsk on Thursday, the Russian RIA Novosti news agency reported." "According to Kolesnikov, the virus of atypical pneumonia is a **synthesis** of the viruses of measles and infectious parotiditis or mumps, the natural compound of which is **impossible**. This can be done only in a laboratory, he said." "Kolesnikov added that in creating bacteriological **weapons**, a protective anti-viral vaccine is, as a rule, worked out at the **same** time, so a medicine for atypical pneumonia may soon appear." "He did **not**, however, rule out the possibility that the virus could have spread accidentally as a result of "an unsanctioned **leakage**" from a biological weapons **laboratory**." Sound familiar? And yet the "epidemic"

was a dud. I would take these duds, and the concomitant warnings of **engineered** pandemic viruses, as further evidence that, if any engineering was going on, it was the "lite" version I've described in reference to **COVID**-19: the duds were **previous** attempts to stitch together the **illusion** of a pandemic—attempts that fell short of success, or were **designed as smaller test runs leading up to what we have now.** The ceaseless propaganda promoting "deadly viruses" is essential to creating the pandemic **illusion**…and sometimes you can see through the **illusion** in graphic terms. Quite, quite clearly. In 1987, a doctor calls me, while I'm writing my first book, **AIDS INC.** He tells me he's built a small AIDS clinic where a group of poverty-stricken patients can rest in clean surroundings, eat nutritious food, and grow beans and sell them for a small amount of money. This doctor is mainstream. He's given his patients no medical treatment. He knows that THE VIRUS, HIV, is said to be a remorseless killer. But, he tells me, **all** his patients have recovered; they no longer have symptoms. They're healthy. He's puzzled, confused, and distraught. He asks me, "What should I do next?" He knows the AIDS drugs are highly toxic. He senses that giving them to his now-healthy patients would bring on a disaster. Oh but you see, according to the **propaganda masquerading as science**, **IT MUST BE THE VIRUS. WHAT ELSE COULD BE CAUSING THESE PEOPLE TO BECOME SICK IN THE FIRST PLACE?** Drinking the water in their villages—water mixed directly

with sewage? Hunger? Starvation? Toxic vaccines pushing their depleted immune systems over the edge of the cliff? Don't be ridiculous. IT MUST BE HIV. In an interview, a famous New York doctor tells me all scientists agree that HIV is the **cause** of AIDS **because**, well, the scientists who **don't** agree **can't** get their findings published. He's telling me all **VISIBLE** scientists agree. Several years ago, during the Zika virus hysteria (another dud epidemic, of microcephaly, that surely would "decimate populations"), researchers in the epicenter, in Brazil, report that only between 10 and 15 percent of Zika patients have any trace of the virus?—they can't find it in the other patients. This amounts to a bald confession that Zika is **eliminated** as the cause of disease in pregnant women. But **no one** listens. **IT MUST BE THE VIRUS. WHAT ELSE COULD IT BE? Well, it turns out it could be ANY INJURY OF ANY KIND TO A PREGNANT MOTHER**—causing her baby to be born with a smaller head and brain damage, called microcephaly. But here, in a **2016** article from thesleuthjournal.com, we have this: "It's [Zika virus] being spread by genetically **engineered mosquitos**. Is it the latest example of US biowarfare? America's sordid history suggests it." If it was being spread in that fashion, it wasn't working to cause disease. It was a **failure**. **But as propaganda, it was a success.** And of course, the World Health organization hit the hysteria button at the time with their **own brand** of propaganda. From marketwatch.com, January 28, 2016:

"The World Health Organization will convene an emergency committee in Geneva on Monday to discuss the **mosquito**-borne Zika virus, which the organization's head said is spreading '**explosively'** and which many doctors and health officials **believe** is linked to an unprecedented outbreak of babies born with small heads in Brazil…'The level of alarm is extremely high,' WHO Director-General **Margaret Chan** said in remarks to the public-health agency's executive board…WHO's announcement underscores the **speed** with which a virus that began as an obscure tropical malady afflicting Africa and then several remote Western Pacific islands has transformed into a major international health concern, particularly in the Americas." Dud. If WHO could squeeze out more **fear**, NOW, in 2020, about an ever-expanding Zika crisis, don't you think they would? Even THEY'VE given up the ghost on that campaign. Meaning: they **achieved their goal of creating alarm and public acceptance of THE VIRUS one more time.** No need to go further for the moment. The **key event in the current COVID** operation was the sudden Chinese government **lockdown** of 50 million citizens overnight in three major cities. That was the signal the CDC and the World Health Organization received with open arms. "Well, they broke the ice. This is what we've been waiting for. This is now a model we can sell. Lockdowns on a massive scale." And they did sell it. As I discovered in 1987, when I was researching AIDS, the basic **epidemic con** involves

grouping all sorts of people and groups who are suffering from different traditional diseases, environmental toxicities, and certain new NON-VIRUS conditions UNDER ONE UMBRELLA LABEL. **And then saying they're all sick because of one virus.** That is the **central** illusion. Finally, I need to make a **general** comment about the effects of viruses on humans. These effects have been **vastly overrated.** Consider the proponents of the so-called "**hot** zone" **hypothesis**. For many years, they've claimed that viruses coming out of rainforests and traveling, in the modern age, to distant countries would cause horrific consequences—in the form of a cascade of **MANY** new diseases. Why? Because the immune systems of people, unacquainted with these **novel** germs, would lack the capacity to ward them off. But that prediction has not come to pass. The hot zone advocates have also failed to mention that the **reverse** vector of travel should also result in massive epidemics: in other words, viruses which are routinely carried by Americans **and** Europeans—and cause them no harm—should be decimating native peoples in rainforests, since the "more civilized" people travel in great numbers **into** jungles. The decimation has not come to pass. Native peoples have been uprooted and damaged by industry, but they haven't been wiped out by American or European viruses. In fact, when you think about it, all countries and locales tend to have their own viruses which are endemic and harmless to locals, but when carried to **other** lands, should be wreaking havoc.

But they aren't. We should all be dead many times over. But we aren't. The hot zone fear stories should also be dead by now. But they **still** attract adherents.

UK Queen makes Kissinger honorary knight
https://www.upi.com/Archives/1995/06/13/UK-Queen-makes-Kissinger-honorary-knight/5808803016000/
https://www.theatlantic.com/politics/archive/2009/03/the-lion-of-the-senate-and-now-a-**knight**/1224/
https://apnews.com/4acd5aad2e114f36411aae36ae6f74ec
https://larouchepub.com/other/2002/2901_**kissinger**.html
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In December of 2005, the **British Medical Journal** (online) published a shocking report by Peter Doshi, which created tremors through the halls of the CDC. Here is a quote from Doshi's report, **"Are US flu death**

figures more PR than science?" (BMJ 2005; 331:1412): "[According to CDC statistics], 'influenza and pneumonia' took 62,034 lives in 2001—61,777 of which were attributable to pneumonia and 257 to flu, and in **only 18 cases was the flu virus positively identified."** Boom. You see, the CDC created one overall category that **combines** both flu and pneumonia deaths. Why do they do this? Because they disingenuously **assume** the pneumonia deaths are complications stemming **from** the flu. This is an **absurd assumption**. Pneumonia has **a number of causes.** But even worse, in all the flu and pneumonia deaths, only 18 revealed the presence of an influenza virus. Therefore, the CDC could not say, with assurance, that more than 18 Americans died of influenza in 2001. **Not** 36,000 deaths. **18 deaths.** Doshi continued his assessment of published CDC flu-death statistics: "Between 1979 and 2001, [CDC] data show an average of 1348 [flu] deaths per year (range 257 to 3006)." These figures refer to flu separated **out** from pneumonia. This death toll is obviously **far lower** than the parroted 36,000 figure. However, when you add the sensible condition that lab **tests have to actually find the flu virus in patients, the numbers of flu deaths would plummet even further. In other words, it's promotion and hype.** "Well, uh, we've said that 36,000 people **die** from the flu every year in the US. But actually, it's probably closer to 20. Who knows? However, we can't admit that, because if we did, we'd be exposing our **gigantic psyop**. The whole

campaign to **scare** people into getting a flu shot would have about the **same** effect as warning people to carry iron umbrellas, in case toasters fall out of upper-story windows…and, by the way, we'd be put in prison for fraud." [Note: **Prior** to Doshi publishing the above piece about flu deaths, I engaged in a series of emails with him about that issue, and independent researcher, Martin **Maloney**, made a major contribution to uncovering the CDC deception.] The **second** big issue is: **how many people diagnosed with the flu really have the flu?** Peter Doshi again, writing in the online BMJ (British Medical Journal), **reveals** another monstrosity. As Doshi states, **every year, hundreds of thousands of respiratory samples are taken from flu patients in the US and tested in labs.** Here is the kicker: **only a small** percentage of these samples show the presence of a flu virus. This means: **most** of the people in America who are **diagnosed** by doctors **with the flu have no flu virus in their bodies.** So they don't have the flu. Therefore, even if you **assume** the flu vaccine is useful and safe, it couldn't possibly prevent all those "flu cases" that aren't flu cases. The vaccine **couldn't** possibly work. The vaccine **isn't** designed to prevent **fake** flu, **unless** pigs can fly. Here's the exact quote from Doshi's BMJ review, **"Influenza: marketing vaccines by marketing disease"** (BMJ 2013; 346:f3037): "…even the **ideal** influenza vaccine, matched **perfectly** to circulating strains of wild influenza and capable of stopping all influenza viruses,

can **only deal with a small part of the 'flu'** problem **because most 'flu' appears to have nothing to do with influenza.** Every year, hundreds of thousands of respiratory specimens are tested across the US. Of those tested, on average 16% are found to be influenza positive." "…It's **no** wonder so many people feel that 'flu shots' **don't** work: for **most** flus, they can't." Because most diagnosed cases of the flu **aren't** the flu. So even if you're a true **believer** in mainstream vaccine theory, you're on the short end here. They're conning your socks off. Let me give you a **gigantic** example of this **massive** flu-case-**counting deception.** It involved a flu "epidemic" you might remember called Swine Flu. In the late summer of **2009**, the Swine Flu epidemic was **hyped** to the sky by the CDC. The Agency was calling for all Americans to take the Swine Flu vaccine. The problem was, the CDC was **concealing** a scandal. At the time, star CBS investigative reporter, Sharyl **Attkisson**, was working on a Swine Flu story. She discovered that the CDC had **secretly stopped** counting US cases of the illness—while, of course, **continuing** to warn Americans about its **unchecked** spread. Understand that the CDC's main job is counting cases **and** reporting the numbers. What was the Agency up to? Here is an **excerpt** from my 2014 interview with Sharyl Attkisson:

Rappoport: In 2009, you spearheaded coverage of the so-called Swine Flu pandemic. You discovered that, in the

summer of 2009, the Centers for Disease Control, ignoring their federal mandate, [secretly] stopped counting Swine Flu cases in America. Yet they continued to stir up fear about the "pandemic," without having any real measure of its impact. Wasn't that another investigation of yours that was shut down? Wasn't there more to find out?

Attkisson: The implications of the story were even worse than that. We discovered through our FOI efforts that before the CDC **mysteriously** stopped counting Swine Flu cases, they had learned that almost **none** of the cases they had counted as Swine Flu was, in fact, Swine Flu or any sort of flu at all!
The interest in the story from one [CBS] executive was very enthusiastic. He said it was "the **most original** story" he'd seen on the whole Swine Flu epidemic. But others pushed to stop it [after it was published on the CBS News website] and, in the end, no [CBS television news] broadcast wanted to touch it. We aired numerous stories pumping up the idea of an epidemic, but **not** the one that would shed original, new light on all the **hype**. It [Attkisson's article] was fair, accurate, legally approved and a heck of a story. With the CDC keeping the true Swine Flu stats **secret**, it meant that many in the public took and gave their **children** an experimental vaccine that may not have been necessary. —**end** of interview excerpt.

It was routine for doctors all over America to send tissue samples from patients they'd diagnosed with Swine Flu, or the "most likely" Swine Flu patients, **to labs** for testing. And overwhelmingly, those samples were coming back with the result: **not** Swine Flu, **not** any kind of flu. That was the **big secret.** That's what the CDC was hiding. That's why they stopped reporting Swine Flu case numbers. That's what Attkisson had discovered. That's why she was shut down. But it gets even worse. Because about three weeks after Attkisson's findings were **published** on the CBS News website, the CDC, obviously in a panic**???**, decided to double down. **If one lie is exposed, tell an even bigger one. A much bigger one.**

Here, from a November 12, 2009, WebMD article is the CDC's response: "Shockingly, 14 million to 34 million U.S. residents — the CDC's best guess is 22 million — came down with H1N1 swine flu by Oct. 17 [2009]." ("22 million cases of Swine Flu in US," by Daniel J. DeNoon). Are your eyeballs popping? They should be. In the summer of 2009, the CDC **secretly** stops counting Swine Flu cases in America, because the overwhelming percentage of lab tests from likely Swine Flu patients shows **no** sign of Swine Flu or any other kind of flu. There is **no** Swine Flu epidemic. **Then**, the CDC estimates there are 22 million cases of Swine Flu in the US. So…the premise that the CDC would **never** lie about

important matters like, oh, a vaccine causing autism…you can lay that one to rest.

The CDC will lie about anything it wants to. It will boldly go where no person interested in real science will go. It will completely ignore its mandate to care about human health, and it will get away with it—as long as people are willing to accept falsehoods instead of the truth, as long as people would rather cling to what authority figures tell them.

And now, with the CDC spearheading the operation called COVID-19—from confirmation of the discovery of a "new virus," to guidelines for diagnostic testing in patients, to case number counts, to containment policies, lockdowns that wreck economies and lives—do you really want to believe what they say?

Corona: the case number game ,by Jon Rappoport March 26, 2020

In this episode of public health bureaucrats go crazy, let's look at their numbers. Let's accept their reality for the moment—the reality they claim to be working from—and trace the implications. Buckle up. Start with Europe and just plain flu. Not COV. **According** to the World Health Organization (WHO) Europe [1], "During the **winter** months, influenza may infect up to 20% of the

population…" That's ordinary seasonal flu. The population of Europe is 741 million people. This works out to 148 million cases of ordinary flu. Not once. **Every year.** According to statista.com [2], "As of **March 23,** 2020, there have been 170,424 **confirmed** cases of coronavirus (COVID-19) across the whole of Europe since the first confirmed cases in France on January 25." I urge readers to roll those comparative figures around in their minds, and realize that ordinary flu has **never** been called a pandemic, and has certainly **never** resulted in **locking** down countries. If we take the COV Europe numbers I just quoted, which cover a period of **two** months, and multiply by six, to estimate the number for a year, we arrive at 1,022,544 cases. Even if you want to build up this figure by claiming it's accelerating, do you really believe it'll reach 148 million for the year, the number of ordinary flu cases? And again, 148 million is the estimate for **Every year-** and **no mention of a pandemic. No lockdowns.**

Let's go to **Italy**. According to statista.com [3], "Italy has the **highest** amount of confirmed [COV] cases in Europe with 59,138…" That's as of **March 23.** If you multiply by six, to get the annual figure, you arrive at 360,000 cases. You want to blow that up, because of acceleration? Go ahead. How about a million cases for the year? Two million. Three million. Now let's look at **ordinary** flu cases for Italy in a given year. According to sciencedirect.com [4], "In the **winter** seasons from

2013/14 to 2016/17, an estimated average of 5,290,000 ILI [influenza-**like** illness] cases occurred in Italy, corresponding to an incidence of 9%." That's 5 million plus each year. **Not** just once. Was a seasonal flu pandemic declared in Italy? Ever? Was the whole country ever locked down as a result? No. Finally, let's look at figures for ordinary flu, for the whole planet. A study published in the journal, Pharmacy and Therapeutics [5], states, "Influenza is a **highly** contagious respiratory illness that is responsible for significant morbidity and **mortality**. Approximately 9% of the world's population is affected **annually**, with up to 1 billion infections, 3 to 5 million severe cases, and 300,000 to 500,000 deaths each year." A **billion** cases **every year.** Is this called a pandemic? Is the whole world locked down every year? No. Feel free to track the purported number of COV cases as time passes. As I write this, the number is 392,286, and deaths are 17,147. Let me know when the COV case number reaches A **billion** for the year and the number of deaths passes 300,000. Then keep me posted on how the one billion COV cases are **repeated** every year with at least 300,000 deaths annually. And that concludes this episode of public health officials go crazy and wreck economies and lives in the process. There should be a tracking "world-o-meter" providing live updates on **THOSE figures. NUMBER OF ECONOMIES WRECKED BY PUBLIC HEALTH LEADERS— NUMBER OF LIVES WRECKED BY PUBLIC HEALTH LEADERS**— Sources: [1].

[Europe] 2018–2019 influenza season: what we know so far [2]. Number of new coronavirus (COVID-19) cases in Europe from January 25 to March 26, 2020, by date of report [3]. Coronavirus (COVID-19) in Italy – Statistics & Facts [4]. Investigating the impact of influenza on excess mortality in all ages in Italy during recent seasons (2013/14–2016/17 seasons) [5]. Influenza Update: A Review of Currently Available Vaccines (P T. 2011 Oct; 36(10): 659-662, 665-668, 684.)

UK downgrades COVID-19 .
No longer a high consequence infectious disease by Jon Rappoport March 25, 2020
Where is the media roar all over the world—blasting out the news that the UK government no longer considers COVID an existential threat to all life on Earth? No giant headlines indicating that the dominos are now starting to fall in another direction—away from sheer suicidal insanity? it's the MEDIA. The UK government, on its website, announced on March 23, under "Status of COVID-19": "As of 19 March 2020, COVID-19 is no longer considered to be a high consequence infectious diseases (HCID) in the UK." UK HCID. "The 4 nations public health HCID group made an interim recommendation in January 2020 to classify COVID-19 as an HCID. This was based on consideration of the UK HCID criteria about the virus and the disease with information available during the early stages of the

outbreak. Now that more is known about COVID-19, the public health bodies in the UK have reviewed the most up to date information about COVID-19 against the UK HCID criteria. They have determined that several features have now changed; in particular, more information is available about **mortality rates (low overall),** and there is now greater clinical awareness and a specific and sensitive laboratory test, the availability of which continues to increase." "The Advisory Committee on Dangerous Pathogens (ACDP) is **also** of the opinion that COVID-19 should **no** longer be classified as an HCID." "The need to have a national, coordinated response remains, but this is being met by the government's COVID-19 response." "Cases of COVID-19 are **no** longer managed by HCID treatment centres only. All healthcare workers managing possible and confirmed cases should follow the updated national infection and prevention (IPC) guidance for COVID-19, which supersedes all previous IPC guidance for COVID-19. This guidance includes instructions about different personal protective equipment (PPE) ensembles that are appropriate for different clinical scenarios."

Here's what the CDC says about the test for the Coronavirus .. Straight from the horse's mouth—both sides by Jon Rappoport March 24, 2020

The CDC (US Centers for Disease Control) admits the coronavirus test is **flawed**. That's the overview and the takeaway— As my readers know, I've described why the widespread diagnostic test for the coronavirus is insufficient, **misleading, useless, and deceptive.** That test, used all over the world where it is available, is called the PCR. It diagnoses patients. "Yes, you have the virus." "No you don't." A very alert reader sent me a link to a US Centers for Disease Control (CDC) document about the test. The CDC establishes the guidelines for how the test should be done, and what the results mean. Here is a CDC paragraph about results. I suggest you read it several times. **"Positive [test] results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.** Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities."

I'm going to blow past the blatant contradiction in that CDC paragraph and cut to the chase. The key line in that paragraph is: "The agent detected [the coronavirus] may **not** be the definite cause of disease.". CDC: Yeah, you see, folks, ahem, the test could say the coronavirus is there in somebody's body, **but the virus may not be causing disease…** On one level, the CDC is admitting the test could turn up false positives: the test could SAY a

patient has the coronavirus, but he really doesn't. This **isn't a footnote** stuck at the bottom of a report. It's right there near the top of the section about the meaning of the test.

CDC is saying straight out, **IF THE TEST SHOWS A CORONAVIRUS IS PRESENT, THAT DOESN'T MEAN IT'S CAUSING DISEASE.**

Well, yes, I've pointed out that the test has an inherent problem. At best, it might show that a virus is present in the patient's body. But the test is incapable of determining **HOW MUCH** virus is **ACTIVELY REPLICATING** in the patient's body. And why is that important? Because, to even begin to say a virus is causing actual illness in a human, there would have to be millions and millions of a virus replicating in his body—and the PCR test has **never** been proven, in the real world, to be able to make such a judgment call accurately. But, if you read that CDC quote again, you'll see the CDC is ordering labs to report a positive test result to public health agencies—where it will be counted as a "coronavirus case". Thank you, CDC. So very, very much.

Coronavirus: **toxic drugs, no liability for Pharma** by Jon Rappoport March 22, 2020
First, we have this, from the World Health Organization (WHO): "There is no specific medicine to prevent or treat

coronavirus disease (COVID-19).” Nevertheless, doctors around the world, often with the **approval** of their **national** governments, are treating many patients with experimental or “off-label” antiviral drugs. Here are some names of the medicines: **Chloroquine, Remdesivir, Ribavirin, favipiravir, lopinavir; ritonavir, hydroxychloroquine, Sofosbuvir, corticosteroids, oseltamivir, zanamivir.** They all have adverse effects. What to do? Answer: decide that no one who is injured by the drugs can file a suit. In America: Done. From druganddevicelawblog.com, March 18, 2020, “We Finally Have Something To Say About COVID-19”: “On March 17, 2020, the U.S. Department of Health and Human Services (“HHS”) published in the Federal Register a ‘notice of declaration’ conferring broad-based immunity from tort (including product liability) litigation for those engaging in ‘activities related to medical countermeasures against COVID-19.’ This declaration is now published at 85 Fed. Reg. 15198 (HHS March 17, 2020).” **“HHS is conferring tort immunity…The immunity extends to ‘any claim of loss , caused by, arising out of, relating to, or resulting from the manufacture, distribution, administration, or use of medical countermeasures’ The immunity extends not only to COVID-19-fighting drugs, but also to ‘products or technologies intended to enhance the use or effect of a drug, biological product [vaccine], or device used against the pandemic’…The only exception is for ‘willful**

misconduct'." "The immunity being conferred shoves other federal laws aside as well as preempting state law."

And that takes care of that. A patient is given an antiviral drug and dies? No law suit can be filed. Anyone associated with the drug, from manufacturer down to prescribing doctor, is exempt from liability. Take one example of a drug, **Chloroquine**. It's approved for the treatment of malaria, and now some doctors are using it on their COVID patients. From webmd.com, here is the "side effects" section (note: once the page loads, then click on the "Side Effects" tab at the top of the page): * "Blurred vision, nausea, vomiting, abdominal cramps, headache, and diarrhea may occur. If any of these effects persist or worsen, tell your doctor or pharmacist promptly." * "Remember that your doctor has prescribed this medication because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this medication do not have serious side effects." * "Tell your doctor right away if you have any serious side effects, including: bleaching of hair color, hair loss, **mental**/mood changes (such as confusion, **personality** changes, **unusual** thoughts/behavior, depression), hearing changes (such as ringing in the ears, hearing loss), darkening of skin/tissue inside the mouth, worsening of skin conditions (such as dermatitis, psoriasis), signs of serious infection (such as high fever, severe chills, persistent sore throat), unusual tiredness,

swelling legs/ankles, shortness of breath, pale lips/nails/skin, signs of **liver** disease (such as severe stomach/abdominal pain, yellowing eyes/skin, dark urine), easy bruising/bleeding, muscle weakness, unwanted/uncontrolled movements (including tongue and face twitching)." * "This medication may rarely cause low blood sugar (**hypoglycemia**). Tell your doctor right away if you develop symptoms of low blood sugar, such as sudden sweating, shaking, hunger, blurred vision, dizziness, or tingling hands/feet. If you have **diabetes**, be sure to check your blood sugars regularly. Your doctor may need to adjust your diabetes medication." * "Get medical help right away if you have any very serious side effects, including: severe dizziness, fainting, fast/slow/irregular heartbeat, seizures." * "This medication may cause **serious eye**/vision problems. The risk for these side effects is increased with long-term use of this medication (over **weeks** to years) and with taking this medication in high doses. Get medical help right away if you have any symptoms of serious eye problems, including: severe vision changes (such as light flashes/streaks, difficulty reading, **complete blindness**)." * "A very serious allergic reaction to this drug is rare. However, get medical help right away if you notice any symptoms of a **serious** allergic reaction, including: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing." * "**This is not a complete list of possible side effects.** If you notice other

effects not listed above, contact your doctor or pharmacist."
No liability. No law suits. No problem. Except for the patient.

The concept of "herd immunity" first materialized in the 1930s, when Johns Hopkins University's Arthur Hedrich discovered that, after 55% of Baltimore's population acquired measles (and thus immunity to measles), the rest of the population, or "herd," became protected. **JOHNS HOPKINS = founded by SKULL & BONES .** This concept provides today's rationale for insisting that everyone be vaccinated. // Herd immunity sounds fine in **theory**. But as Stanford's Dr. Obukhanych concluded, "As with any garbage in-garbage out type of theory, the expectations of the herd-immunity **theory** are bound to **fail** in the real world."
https://business.financialpost.com/opinion/junk-science-week-vaccinating-the-herd She begins by defining the term, and orienting us to the fact that "herd immunity" is **not a scientifically validated concept, let alone one that applies definitively, predictably, or preventatively to vaccinated communities: Herd immunity is not an immunologic idea, but rather an epidemiologic construct, which theoretically predicts successful disease control when a certain pre-calculated**

percentage of people in the population are immune from disease.
https://kellybroganmd.com/herd-immunity-fact-fiction/
https://childrenshealthdefense.org/news/herd-immunity-a-false-rationale-for-vaccine-mandates/
https://www.wakeupuk.net/why-herd-immunity-is-a-hoax/
www.imusenvironmentalhealth.org/why-herd-immunity-is-a-hoax/
https://science.news/2018-01-30-flu-vaccine-bombshell-630-more-aerosolized-flu-virus-particles-emitted-by-people-who-received-flu-shots-flu-vaccines-actually-spread-the-flu.html

No Virus. No Herd Immunity. Humans Cant Survive Everywhere-Desert/Ice Mountains. It Rains Where Plants Grow-or its the Other Way.

WAS THERE A PROVEN EPIDEMIC / PANDEMIC -- WHAT PROOF DO WE HAVE ?? there could be many diseases restricted to childhood . because of the childhood habits , food or their physiology . when they become adults they become stronger . its doesnt mean they acquired immunity , because they had that disease when they were children .

Arthur William Hedrich, American public health service officer Member **Alpha Chi Sigma, Phi Beta Kappa,**

Delta Omega.
https://prabook.com/web/arthur_william.hedrich/1045853
https://truthsnitch.com/tag/arthur-hedrich/#sthash.wJOWwUMV.dpbs
https://www.spiritofchange.org/alternative-health/The-Misunderstood-Theory-of-Herd-Immunity/

we dont have a properly developed system in childhood . not prepared to handle many situations . we dont know much about ourselves

http://duluthreader.com/articles/2019/12/12/19019_why_you_cant_trust_the_fda_the_who_the_cdc_the_aap
https://www.globalresearch.ca/hospitals-getting-paid-more-label-cause-death-coronavirus/5709720

US data on influenza deaths are false and misleading. The Centers for Disease Control and Prevention (CDC) **acknowledges a difference between** flu death and flu **associated** death **yet uses the terms interchangeably.** Additionally, there are significant **statistical incompatibilities** between official estimates and national vital statistics data. Compounding these problems is a **marketing of fear**—a CDC communications strategy in which medical experts "predict dire outcomes" during flu seasons.

https://aspe.hhs.gov/cdc-—-influenza-deaths-request-correction-rfc

CDC uses a mathematical model to estimate
https://www.cdc.gov/flu/about/burden/**how-cdc-estimates**.htm
https://aspe.hhs.gov/cdc-%E2%80%94-**influenza-deaths-request-correction**-rfc
https://steemit.com/health/@johnblaid/**the-existence-of-any-virus**
https://chinadigitaltimes.net/2019/07/**thousands-in-wuhan-protest-against-waste-incineration**
https://www.researchgate.net/publication/281876323_Why_the_Corruption_of_the_**World_Health_Organization_WHO_is_the_Biggest_Threat_to_the_World's_Public_Health_of_Our_Time**

Janet Godfrey
Clap! Clap! Clap! The PR Campaign
Will the real Annemarie Plas please stand up!

The UK COVID19 death toll has reached over 11,000 and the nation is gripped with fervour and appreciation for our wonderful NHS. Every Thursday we're told to go outside and clap, bang saucepans, play music, cheer for care workers. What a wonderful grassroots movement this is….or is it?

Who started the campaign?
Annemarie Plas is the “yoga teacher/ something in sales” who apparently came up with the idea one night, while having a virtual drink with friends. She says she knocked up a graphic, adding the NHS logo, and put it out on social media. Hey presto it took off!

But Annemarie is surely selling herself short. You see, although she is a certified yoga teacher, it’s not her day job. She is in marketing, or more accurately she’s the Regional Sales Director at Objective Partners, a Netherlands based company, who set up a London office in October 2019. Annemarie moved to London to head up the office, along with Rudy De Back, the CEO. I wonder why she didn’t mention this in interviews.
(https://www.linkedin.com/in/annemarieplas)

Objective Partners specialises in data analytics for sales and marketing. If you don’t understand all that jargon, they’re a bit like Cambridge Analytica. Remember them?
https://objectivepartners.com/improve-data-quality

So back to Annemarie, the “yoga teacher” who had a Eureka moment, wanted to thank carers and scribbled a graphic to share on social media. She says she put out a graphic urging everyone to clap, and that it included the NHS logo. This is surprising because, as someone experienced in branding, Plas would have been aware that

using a branded logo, without permission, would be a serious breach of copyright law. So, either she didn't know (highly unlikely or seriously negligent) or she knew but she did it anyway, or possibly she had prior permission. In a hugely bureaucratic organisation, like the NHS this would surely take months to obtain.

PR agency Hue & Cry say they offered to help Plas by "reaching out to news publications on her behalf and giving her a bit of media training". A Sales Director of a media and PR company needed help with the media. Who'd have thought?
https://www.creativereview.co.uk/clap-for-our-carers

An Absolute PR Godsend for Government
Amidst an escalating COVID19 death rate, now over 11,000, serious lack of protective equipment for our frontline workers and criticism of government from the BMA, Nursing bodies and the Lancet, British people clap for all their worth on Thursday nights to show their appreciation" for care workers who are literally dying on the job. The Clap for Carers initiative couldn't have come at a better time.

On the 28 March the editor of prestigious medical journal The Lancet hit out at Boris Johnson's government over the way it has handled coronavirus, branding it a "national scandal".

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30727-3/fulltext

But on the 26 March the British public was already being urged to put politics aside and support our carers. Television and online media showed photos and videos of nationwide streets and apartment blocks, filled with cheering, clapping residents, while people died quietly in care homes.

Now the public is asked to help raise £5million for NHS charities.On the back of the Clap for Carers initiative, Organisers of the "One Million Claps" appeal are asking for at least 1 million people to donate £5 by text message to fund food deliveries, overnight stays, kits and travel costs.

Plas is at pains to point out that she "doesn't work for the government or the NHS" but if government spin doctors wanted a way to deflect from their own failings, brush aside the fact that the Tories have been dangerously underfunding the NHS for the last decade, and that they refused to update the Pandemic Plan, as advised by NHS scientists in 2016, her Clap for Carers campaign is a godsend.

Not only does it take our eye off the ball when it comes to the pitiful failure to fund and staff the NHS but now,

we’re expected to top up the shortfall. The beauty of this is government can continue to claim it’s a party of low taxation, while taxing the poor again. “Come on who would begrudge a fiver for the lovely NHS in a time of crisis? And this is all given wholehearted support by multimillionaire, tax-dodging celebs.

Simple Slogans

Once again it appeals to the public’s emotions. They don’t want to get bogged down in lengthy discussions about underfunding and privatisation of health and social care services or the multimillion-pound profits made by large national companies running care homes, failing to provide staff with PPE. They want a simple, positive message, a slogan “Clap for Carers” a bit like “Get Brexit Done!” I mean who could argue with it? We all love the NHS and we are immensely grateful for the work all key workers do (the erstwhile low-skilled, not required when it comes to Priti’s immigration policy)

And what if you don’t want to clap?

As the nation turns into bored curtain-twitchers, thanks to ambiguous guidelines about what are essential items and whether we can sit on a park bench, you might well find the local Neighbourhood Watch representative pays you a visit to ask why you’re not joining in the clap. An ex-nurse and Neighbourhood Watch rep told how she

knocked on her neighbour's door but was "really upset" when he wouldn't join in. What next..white feathers? https://www.express.co.uk/news/uk/1261266/Clap-for-NHS-video-nurse-coronavirus-uk-latest-covid-19-news

If a Machiavellian special advisor wanted to devise a campaign to bring the country together, in a collective focus away from government failure, one that all sides of the political spectrum would feel obliged to get behind, Cummings couldn't have done a better job than Annemarie, the "yoga teacher".
…Donna Gardner

he doesnt say , viruses dont exist .. otherwise its a lot of info https://www.youtube.com/watch?v=oWBRNwoUqFY
https://healthrecoverysecrets.wordpress.com/2017/04/19/vaccines-cause-diseases

time 6.55 mask
https://www.youtube.com/watch?v=3RVG8qNLdoY
https://www.youtube.com/watch?v=EHwZRMg64Ow
http://www.shotsoftruth.com

US virus numbers now include probable cases without tests
https://apnews.com/dea52e0a0eb179d51ac5eed99a99bf10

Gates Foundation calls for global cooperation on vaccine for 7 billion people https://news.yahoo.com/gates-foundation-calls-global-cooperation-vaccine-7-billion-223450593.html

Children could be forced to have compulsory vaccination under Government plans
https://metro.co.uk/2019/09/30/children-forced-compulsory-vaccination-government-plans-10830352/amp

RHODES SCHOLAR
https://www.jagranjosh.com/current-affairs/indian-origin-scientist-ss-vasan-leads-a-team-to-develop-coronavirus-vaccine-1581143764-1
https://www.mckinsey.com/alumni/news-and-insights/global-news/alumni-news/ss-vasan-developing-coronavirus-vaccine

covid19=bioweapon.can be "contagious"?.requires BIG govt. dr.shiva=MIT/tavistock.promotes vit d3-rodent poison.plays at low level
fauci giving wrong advises to trump ?? trump is innocent / stupid ? fouci the only person trump can take advises from ?
https://www.youtube.com/watch?v=86VJlhw0DQQ

https://www.wma.net/who-we-are/alliance-and-partner/partners/

HIV does not cause AIDS.... The point that everyone is missing is that all of those original papers Gallo wrote on HIV have been found **fraudulent**.... The HIV **hypothesis was based on those papers.** — Peter Duesberg
https://www.youtube.com/watch?v=y2Q0rpnXq7Q
https://en.wikipedia.org/wiki/Robert_Willner
www.whale.to/c/willner.html
www.whale.to/c/willner_deadly_deception.html
https://www.dailymotion.com/video/x2r4rqo
https://www.youtube.com/watch?v=e5rOZar23Og

NO VIRUS https://steemit.com/health/@johnblaid/the-inconvenient-facts-about-the-coronavirus-pandemic
https://www.rushlimbaugh.com/daily/2020/04/08/death-model-reduced-90-since-we-heard-2-2-million/

To all the soldiers and police officers & MEDICAL who will participate in forcibly removing people from their homes...what do you think they are going to do to you when they don't need you any more? This is what the controllers think of you...

“Military men are just dumb, stupid animals to be used as pawns in foreign policy.”
— Henry Kissinger

LET THAT SINK IN...

THEY claim a virus w/out evidence, THEY invented the tests w/out proof, THEY dictate treatments. THEY are openly psycho.

Lock Down = Strict Observance Rite. similar to 1782 wilhelmsbad illuminati conversion the whole of german freemasonry into SOR.

The Wilhelmsbad Congress was held in the summer home of William I of Hanau on July 16, 1782. It was about this same time that Jews were first allowed to enter Freemasonry. William later became William IX, Landgrave of Hesse-Kassel. William eventually named Mayer Rothschild to handle his financial affairs.
www.travelingtemplar.com/2013/07/the-1782-congress-of-wilhelmsbad.html
https://www.conspiracyarchive.com/2015/07/06/masonic-congress-of-wilhelmsbad/
https://www.spencorp.info/rothschild-family
https://newworldorderuniversity.com/wilhelmsbad-convention-1782
https://amallulla.org/illuminati
http://www.mymind.info/complete-history-of-the-illuminati-by-robert-morningstar-and-the-late-jim-marrs

cdc , fda,who = all run by same people .they say , they know nothing about cv

people are different . changes in seasonal climate affect . different people differently . & there is NO virus dead or alive . no one has seen any

Dr. Fauci: Coronavirus could become seasonal, unlikely to be under control globally
https://abc7chicago.com/health/fauci-coronavirus-could-become-seasonal/6078871

Louise Winkler.... My niece is an ER nurse in a hospital in north central Florida. They have been so NOT busy that they are canceling nurses who are scheduled to work. I drove by my area hospital in Florida, and there was no sign of any emergence. There was an open tent in front of the ER, staffed with one nurse who was chatting with someone, and one EMT parked. Very quiet.

Jimi Mo .... that's pretty much the same situation shown in these links. Empty Hospitals Corona Scam-Demic
https://youtu.be/wElEJqAWiMg
https://youtu.be/H1fm1VQgX8M
https://youtu.be/jpc-Jyk9uF4
https://www.facebook.com/623971295/posts/10156720677816296/?d=n
https://youtu.be/ydIz7ldbJH0?t=1018

https://youtu.be/yb92TXYMN-4
https://youtu.be/KyemBXp_1Lg
https://youtu.be/aVFC5n-KnuA
https://www.youtube.com/watch?v=K0z8NhxNTaU
https://www.youtube.com/watch?v=cNVTNNmurgA
https://m.youtube.com/watch?v=anVwA1QWS24
https://youtu.be/VuUjzcmnylk
https://youtu.be/p-O_UVMqWWA
https://youtu.be/Uuy6r6OkFKc
https://youtu.be/obukMhlcjs0
https://youtu.be/E2r-JEyxCZA

Rikke My.. to FILM YOUR HOSPITAL
Hello from Denmark!
Our hospitals are also empty - just not according to the news .
Our Corona deaths are counted, including all deaths - even heart attacks and traffic accidents - if they have been tested positive within 60 days before their death.
Our country is in lockdown.
Our government is overruling 200 years old laws - even the constitusion.
We officially have no freedom of speech, since you can now get up to 8 months in prison, for writing against the government's recommendations.
Our economy is FUCKED!

They have already said that nothing is going to be the same again.

Mikey Deuce.. Same situation here in Norway // Mikey Deuce ...Brian Porter ..in Sweden everything is open haha. Malls, gyms, schools etc. They are taking a different approach // Mikey Deuce.. My friends girlfriend works at the largest hospital in my city. Media claims its packed. She says its quiet // Brian Porter ..Sweden is the "control" in this worldwide experiment i guess // Kay Richards-Stapley ..Brian Porter.. The numbers are for fear mongering only.. // Marie E. Lockwood ..Mikey Deuce ..They are already cashless and chipped in Sweden. // Mikey Deuce.. Marie E. Lockwood ..only a small group who wanted it themselves. Its in no way mandatory at this point // Marie E. Lockwood ..Yes the chips were voluntary but the society is mostly cashless. Even most of their banks no longer do any cash transactions. // John Thomas... Milhorat Rikke , I am from New York and everything you described is going on here as well. We are all in the same boat it seems. Hang in there my friend. As long as more people begin to realize what is really going on and how we have been all brutally lied to all over this Earth , there is a chance to stop this madness. At least, I like to think so. //

https://www.facebook.com/KevinMugurGalalae/posts/2580653932176222
https://www.academia.edu/42632701/Evidence_the_COVID-19_pandemic_is_false
https://www.medicalnewstoday.com/articles/coronavirus-testing

There is NOTHING called VIRUS . medically relevant / pathogenic.

Even in the latest version coronavirus , strange to be a **single** word - it means something else , they are **not** identifying the so called virus itself , **but** something THEY CALL ncov-2019 RNA.

study the protocols / processes involved in PCR whatever version , RT or Q RT . its worse than assumptions and presumptions , recklessness , conspiracies , very crude but given complex sounding names purely to deceive.

Virus is Political.

final declaration involves INTERPRETATION . and DEFINITION can create sickness / pandemic .
PCR is the MOST SENSITIVE / POPULAR -- it does NOT mean accurate / precise. without a precise PCR or whatever , without isolating a purified virus , how do they come up with ANTIBODY tests? many will be

SACRIFICED. how PCR became most popular . they made it popular .

Regarding the current coronavirus pandemic, they said: "If one assumes that the number of asymptomatic or minimally symptomatic cases is several times as high as the number of reported cases, the case fatality rate may be considerably less than 1%."

New WUHAN study shows deaths to be between 0.04 to 0.12% This is 33 times lower than the media headlines. -- WE SEE TOO MANY 33 EVERYWHERE RE covid19 ... google --- 33 coronavirus

Dr. Fauci is also the head Director at the National Institutes of Health for the Gates foundation. Fauci is pushing the whole country to hurry and get the vaccines when they are out. We also see where the COVID-19 vaccine is heading. **They are using fear tactics off the deaths to mandate it.**

Bill Gates Calls For National Tracking System For Coronavirus During Reddit AMA
https://www.forbes.com/sites/mattperez/2020/03/18/bill-gates-calls-for-national-tracking-system-for-coronavirus-during-reddit-ama/

https://www.forbes.com/sites/simonchandler/2020/03/23/

coronavirus-could-infect-privacy-and-civil-liberties-forever
https://www.nejm.org/doi/full/10.1056/NEJMe2002387
https://annals.org/aim/article-abstract/2762506/effect-influenza-vaccination-elderly-hospitalization-mortality-observational-study-regression-discontinuity
https://www.medrxiv.org/content/10.1101/2020.02.12.20022434v2

Media's hysteria and the politicians' rhetoric is going pandemic.

German immunologist and toxicologist, Professor Stefan Hockertz, explains in a radio interview that Covid19 is no more dangerous than influenza (the flu). More dangerous than the virus is the fear and panic created by the media and the „**authoritarian** reaction“ of many governments.

Professor Hockertz also notes that most so-called „corona deaths“ have in fact died of other causes while also **testing positive** for coronaviruses.

SYMPTOMS MEAN NOTHING ANYMORE. THE SCIENCE ESTABLISHMENT WILL DECIDE , WHO HAS WHAT , WHETHER HE SHOULD BE CULLED OR QUARANTINED .

The Argentinean virologist and biochemist Pablo Goldschmidt speaks of a „global terror“ created by the

media and politics. Every year, he says, three million newborns worldwide and 50,000 adults in the US alone die of pneumonia.

Professor Julian Nida-Ruemelin, former German Minister of State for Culture and Professor of Ethics, points out that Covid19 poses no risk to the healthy general population and that extreme measures such as curfews are therefore not justified.

Using data from the cruise ship Diamond Princess, .. the age-corrected lethality of Covid19 is between 0.025% and 0.625%, i.e. in the range of a strong cold or the flu.

Moreover, a Japanese study showed that of all the test-positive passengers, and despite the high average age, 48% remained completely symptom-free; even among the 80-89 year olds , 48% remained symptom-free, while among the 70 to 79 year olds it was an astounding 60% that developed no symptoms at all.

The Italian example has shown that 99% of test-positive deaths had one or more pre-existing conditions, and even among these, only 12% of the death certificates mentioned Covid19 as a causal factor.

“Coronavirus is a virus with public relations”
— Prof. Yoram Lass, MD

https://www.youtube.com/watch?v=MZcLqcoL49M

https://www.facebook.com/northerntracey/posts/1021676916968353 6
https://greatgameindia.com/who-vaccine-industry
https://www.youtube.com/watch?v=dvKn4OzcTzM
https://www.fda.gov/media/136151/download

Coronavirus test crisis as kits shipped in from Europe found contaminated with COVID-19
https://www.express.co.uk/news/uk/1262588/UK-coronavirus-news-boris-johnson-covid-19-testing-kits-death-infection-rates-latest
https://www.fda.gov/media/136151/download
http://activehealthcare.co.uk/index.php/literature/medical/208-medical-errors-do-not-go-viral
https://www.youtube.com/watch?v=_dslfuSBY2U

“A really efficient totalitarian state would be one in which the all-powerful executive of political bosses and their army of managers control a population of slaves who do not have to be coerced, because they love their servitude.”
— Aldous Huxley, Brave New World

https://www.youtube.com/watch?v=E1wIsMi8ryw
https://www.ncbi.nlm.nih.gov/pubmed/23860193

https://www.youtube.com/watch?v=5pIMD1enwd4
https://www.cnbc.com/2020/03/30/sweden-coronavirus-approach-is-very-different-from-the-rest-of-europe.html

“This concern with the basic condition of freedom — the absence of physical constraint — is unquestionably necessary, but is not all that is necessary. It is perfectly possible for a man to be out of prison and yet not free — to be under no physical constraint and yet to be a psychological captive, compelled to think, feel and act as the representatives of the national State, or of some private interest within the nation, want him to think, feel and act.” **— Aldous Huxley,** Brave New World

some people in coronavirus drama

attendees included **IRAN FOREIGN MINISTER MOHAMMED JAVAD ZARIF**

Gualtiero Ricciardi , known as Walter Ricciardi (born 1959), is an Italian doctor and ACTOR.

Sunetra Gupta is a NOVELIST , and Professor of THEORETICAL Epidemiology at the University of Oxford with an interest in infectious disease agents that are responsible for malaria, HIV, influenza and bacterial meningitis.”My main area of interest is the evolution of

diversity in pathogens, with particular reference to the infectious disease agents that are responsible for malaria, influenza and bacterial meningitis. I use simple MATHEMATICAL MODELS to generate new HYPOTHESES regarding the processes that determine the population structure of these pathogens. I work closely with laboratory and field scientists both to develop these HYPOTHESE and to test them.
https://www.zoo.ox.ac.uk/people/professor-sunetra-gupta

https://m.washingtontimes.com/news/2020/mar/26/**lockdown-britain-quietly-downgraded-status-virus-t**

there is NOTHING called ELECTRON . we have electronics engineering . but dont deal with electron . EM is a huge machine . ONE END they put the adulterated , manipulated , cooked , cooled , mixed up , so called sample , which is supposed to contain the contagion , with too many heavy chemicals .... BASED on double helix model ,"invented " by rosalind franklin directly from rothschild family . which was stolen by freemasons , THEORETICAL scientists crick & watson , who were into sex orgies ------ OTHER END they show you some picture and claim it to be picture of virus . --- do we have proof , when they add RT , rna is getting converted into dna ??

There is NO confirmatory tests to prove the existence of ANY viruses . Where is the SCIENTIFIC PAPER ?.
2 covid 19 also is not genuinely proven.
A virus attacks , the body produces antibody . if virus is properly not identified & isolated , how the antibody of that virus is proven to be genuine ? where is the scientific paper?

https://revealingfraud.com/2020/03/health/35-sources-the-covid19-corona-virus-is-over-hyped-and-likely-fraud/
https://pieceofmindful.com/2020/03/16/coronavirus-panic-is-mass-hysteria-and-nothing-more/
https://www.bmj.com/content/368/bmj.m627/rr-14
https://www.theglobeandmail.com/opinion/article-strictly-by-the-numbers-the-coronavirus-does-not-register-as-a-dire/
https://www.continentaltelegraph.com/health/what-if-coronavirus-is-just-the-old-mans-friend-pneumonia/
https://www.thegatewaypundit.com/2020/03/breaking-italian-health-ministry-confirms-only-twelve-coronavirus-fatalities-did-not-have-other-serious-health-complications/
https://freerepublic.com/focus/f-news/3825709/posts
https://www.unz.com/isteve/my-new-takimag-column-reasons-for-hope/

reasosns for sickness vaccines , chemicals , malnutrition , uncleanliness , not understanding their particular body types - susceptibility to seasonal change in climate . and unknown reasons

http://www.whale.to/vaccine/sf1.html
https://news.yahoo.com/chinas-coronavirus-recovery-fake-whistleblowers-191300391.html
Coronavirus: Social distancing may need to go on for almost 12 months, UK's scientific advice says
https://www.independent.co.uk/news/health/coronavirus-uk-self-isolation-cases-social-distancing-advice-boris-johnson-a9413836.html

(THIS GUY IS THEIR MAN) Walter Ricciardi, a member of the World Health Organization's executive council and Italian health ministry consultant on the coronavirus, provided a modest timeline last week. Ricciardi suggested life could return to "normal" this summer. Ricciardi compared the coronavirus pandemic to the SARS outbreak almost two decades ago, which he said ended in May or June. "I have the impression that, if we are lucky and all work together, we should get through to the summer," he said. "That's when we should be able to return to normal life."
https://www.usatoday.com/story/news/health/2020/03/15/

coronavirus-crisis-end-summer-experts-odds-what-we-dont-know-epic/5053876002/

Cowie made the statement in a scientific paper that the etiological agent of poliomyelitis is unknown, .. and in the recent book, An American Doctor's Odyssey, Heiser remarked that "the microbe which causes smallpox has never been discovered."

https://blog.nomorefakenews.com/2020/03/13/italy-icu-wards-are-overflowing
https://www.sciencedirect.com/science/article/pii/S1201971219303285

There are no reliable tests for a specific COVID-19 virus. There are no reliable agencies or media outlets for reporting numbers of actual COVID-19 virus cases. This needs to be addressed first and foremost. Every action and reaction to COVID-19 is based on totally flawed data and we simply can not make accurate assessments.

http://alternativehealthadvice.blogspot.com/2020/03/no-one-has-seen-living-virus.html
www.shotsoftruth.com
https://en.wikipedia.org/wiki/History_of_virology
https://www.bing.com/images/search?q=dr+mendelsohn+on+vaccines&FORM=QBIR

https://www.youtube.com/watch?v=t0uemkf9c88

there is NO virus . people falling sick / dying -- look for the cause somewhere else . susceptibility to changes in climate , toxic medicines & chemicals , lack of nutrition , biological warfare , mental stress ... ... but dont call it virus
https://swprs.org/a-swiss-doctor-on-covid-19/
https://www.worldometers.info
http://deathmeters.info/

Every year about 15 million people die from respiratory ailments. Even conservative estimates say the flu kills over 300,000 people annually. And yet here we have extreme hysteria over a few thousand deaths of people whose average age is 80-years old.

“The extremely long list of celebrities and first ladies (Spain, Canada) all coming out to announce that they allegedly have Covid-19. So many in fact it’s absolutely ludicrous, considering that they never frequent the subway or mingle with the unwashed and the deplorable. The list included over 157 people, which doesn’t compute with the true statistics of non-celebrities diagnosed with it.”

http://theocs101ark.com/2020/03/17/dont-get-locked-down/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6815659
http://healthinsightuk.org/2020/02/12/coronavirus-a-reliable-test-is-badly-needed-we-dont-have-one/
https://abruptearthchanges.com/2020/03/04/coronavirus-**low-accuracy-rate-of-tests-but-high-global-government-control/**
https://abruptearthchanges.com/2017/11/17/**dr-stefan-lanka-the-history-of-the-infection-theory/**

electrons , protons , neutrons are parts of atom .no one has seen any atoms or molecules. its occult mysticism .. cathode rays CONSIST of negatively charged particles called ELECTRONS.

MIND IT . i am NOT supporting this man . ONLY his views on electron & EM . he is pro tesla & against einstein ------ . i am against tesla & einstein...
https://www.youtube.com/watch?v=dnEx3JjeGGg
https://www.youtube.com/watch?v=y8hein2SDrk
https://www.youtube.com/watch?v=Bz_0UM1IxvI
https://www.youtube.com/watch?v=NS6TLX8RTa4

its THEORETICAL science . occult chemistry . all these guys are THEORETICAL scientists
not logical . they say atoms are made up of electrons / protons / neutrons . they are specific . without proof . if

they called it cathode ray microscope , or Y ray ... it would be ok . to some extent . /// result of x ray is verifiable . result of EM is NOT verifiable

https://www.bmj.com/content/337/bmj.a2398

The Secretive Group Guiding the U.K. on Coronavirus

Mark Landler and Stephen Castle

The government is planning to have 50 drive-through testing sites by the end of April. Key workers can now order coronavirus tests online from today Virus test for millions of key workers: Today's front pages The Houses of Parliament in London. The government has deflected pressure to identify the members of its scientific advisory board.© Andrew Testa for The New York Times The Houses of Parliament in London. The government has deflected pressure to identify the members of its scientific advisory board.

As the British government comes under mounting criticism for its response to the coronavirus — one that has left Britain vying with Italy and Spain as the worst hit countries in Europe — Prime Minister Boris Johnson and his aides have defended themselves by saying they are "guided by the science." The trouble is, nobody knows what the science is. The government's influential Scientific Advisory Group for Emergencies — known by

its soothing acronym, SAGE — operates as a virtual black box. **Its list of members is secret, its meetings are closed, its recommendations are private and the minutes of its deliberations are published much later, if at all.**

With all the secrecy, even some of Britain's top scientists say they don't know whether they can trust the government's approach. "Is the science being followed by the government on coronavirus?" said David King, a former chief scientific adviser to the government. "I don't know, because I don't know what the advice is, and there isn't the freedom for the scientists to tell the public what their advice is."

 Professor King, who counseled Prime Minister Tony Blair on the foot-and-mouth disease that infected British farm animals in 2001, said there was no justification for the government to withhold either the advisory group's membership or the minutes of its meetings. Doing so, he said, eroded public trust in the government, especially given the bewildering twist and turns in its response. **It also raises questions about an academic group that** ought to be a point of pride for Britain: the country's best scientific minds, in fields from epidemiology to behavioral science, assembled from cutting-edge labs at Cambridge, Oxford, Imperial College and the London School of Hygiene and

Tropical Medicine. a group of people standing in a parking lot: Lining up outside a supermarket in London. “The names are likely to come out at some stage,” said David Lidington, who served as a deputy to Mr. Johnson’s predecessor, Theresa May. He warned that the government’s lack of disclosure would only cause more headaches later. “There is the risk that if names leak out after a time it becomes a great shock-horror,” he said, adding that it would be better to make a virtue of transparency. Even now, outside scientists and doctors are second-guessing the advice of these unnamed authorities. Why, for example, did SAGE recommend less stringent social-distancing measures on March 9, when France and Ireland were banning large events and ordering lockdowns, and there was ample evidence from Italy of the epidemic’s rapid and lethal spread? (The unusual disclosure came in a report the government posted about the predicted effects of various social-distancing measures.) a person standing in front of a building: Workers at the Nissan car plant in Sunderland packaging up personal protective equipment to send out to the N.H.S.

Workers at the Nissan car plant in Sunderland packaging up personal protective equipment to send out to the N.H.S. Why in late February did a subgroup of SAGE experts underestimate the percentage of people who would have to be hospitalized as a result of contracting the virus, and why did their models underestimate the

speed at which the pathogen spread? Why did those scientists agree to classify the risk level of the contagion to the public as “moderate,” even after weeks of evidence that it was being transmitted between humans in China? Why, after Imperial College London published a frightening study on March 16 that projected up to 500,000 deaths if Britain did not act more aggressively to curb the virus, did Mr. Johnson wait another full week to close nonessential shops and order people to stay in their homes? “Political decisions are often framed as following the best scientific advice,” said Connor Rochford, a physician and former consultant at **McKinsey &** Company. “But science is nothing more than a normative claim about how we ought to make a decision. These are best-guess estimates.” Some said the frequent references of Mr. Johnson and his aides to the scientists should be a warning sign. If, as is likely, the government’s handling of the crisis is scrutinized in a future parliamentary inquiry, officials are likely to justify their actions by saying they were listening to the experts. Messages supporting N.H.S. workers in Blackhall Colliery, one of Britain’s poorest areas.

Messages supporting N.H.S. workers in Blackhall Colliery, one of Britain’s poorest areas. “It has become a shield for them,” said **Devi Sridhar,** director of the global health governance program at Edinburgh University. **“If things go off, you can always say, ‘Well, it was the experts who told us.’”** The government has deflected

pressure to identify the group's members or how many there are by noting that Patrick Vallance, the current chief scientific adviser, who chairs the group, regularly appears in public at news conferences. The government also posts brief reports from some of SAGE's subgroups, and the data that go into its models, on the internet. In a recent letter to Parliament, Professor Vallance said **anonymity** protected the security of scientists and also shielded them "from lobbying and other forms of unwanted influence which may hinder their ability to give impartial advice." He added that people were free to disclose their membership. One member who has — Jeremy Farrar, an infectious disease specialist who is the **director of Wellcome Trust** — acknowledged the limitations of the system when he recently told the BBC that the New and Emerging Respiratory Virus Threats Advisory Group, which advises SAGE, underestimated the threat of the contagion in March. "The U.K.," Dr. Farrar added, "is likely to be certainly one of the worst, if not the worst, affected countries in Europe."

Jeremy Farrar Another member who has become a household name, and a source of scrutiny for his eye-watering statements, is **Neil Ferguson, an epidemiologist at Imperial College London.** His team of modelers produced the March 16 report that prompted Downing Street to impose a lockdown (it was also influential at the White House, which embraced social distancing). Professor Ferguson, who collaborates with the World

Health Organization and has advised other countries on how to deal with epidemics, later came down with symptoms of the virus himself. In late March, testifying before Parliament from self-isolation in his house, he generated more headlines when he said that Britain could keep its death toll under 20,000 if it stuck with strict social distancing. Professor Ferguson did not reply to requests to discuss his advice to the government or the deliberations of SAGE. But in an interview with The New York Times the day the March 16 report was published, he laid out the choice Britain faced: Manage the spread of the virus in a way that minimized deaths but allowed a significant percentage of the population to become infected — a situation known as **“herd immunity.”** Or tamp down transmission of the virus by imposing a lockdown of the kind the Chinese government did in Wuhan. In the end, he said, there was no choice but to take the latter course. “The U.K. has struggled in the past few weeks in thinking about how to handle this outbreak long term,” Professor Ferguson said. **“We don’t have a clear exit strategy,** but we’re going to have to suppress this virus, frankly indefinitely, until we have a vaccine. It’s a difficult position for the world to be in.” Until mid-March, Professor Ferguson, Professor Vallance and other scientists had appeared receptive to the case for “herd immunity.” Then, confronted with new numbers that projected hospitals would be overwhelmed with patients and that the death toll would skyrocket, they pivoted to a

suppression strategy. What is unclear is the role SAGE played in shifting the government's thinking. a group of people walking in front of a store: In late February a subgroup of SAGE experts underestimated the number of people who would have to be hospitalized as a result of contracting the virus.

In late February a subgroup of SAGE experts underestimated the number of people who would have to be hospitalized as a result of contracting the virus. One of the few public documents that gives a glimpse into its deliberations — a March 9 report assessing the potential impact of social distancing measures — said the group recommended "a combination of individual home isolation of symptomatic cases, household isolation and social distancing of the over 70s." That is far short of the lockdown measures Britain ultimately adopted. It did not, for example, include a ban on large gatherings, like concerts and sporting events, in part because behavioral scientists doubted there would be enough compliance with the bans to reduce the spread of the virus. Nor did it include a recommendation for widespread testing and contact tracing of people who had contracted the virus — a policy the government had pursued with some success during the earliest days of the outbreak in Britain. "I'm guessing there was a debate between containment and mitigation, and they decided to go with Option B," Professor Sridhar said. But she added there was no way to be sure until the deliberations were made public. Among

the other mysteries of SAGE is the makeup of the group. Professor Vallance said it includes representatives from more than 20 institutions, with expertise ranging from molecular evolution to microbiology. There are four expert groups, with anywhere from five to 45 members, whose advice is funneled into SAGE. Some scientists, like Professor Ferguson, serve on multiple panels.
Gallery: How the coronavirus is being handled globally
Britain's lack of masks, gloves and other protective gear has become another weak link in its response. Some experts also said the scientists suffered from a lack of independence. While Professor Vallance has begun to show some daylight with the government — he recently said SAGE would re-examine the government's decision not to advise people to wear masks — his regular public appearances next to Mr. Johnson and other cabinet ministers have made him look too much like an agent of the government rather than an independent adviser, according to critics. On Thursday, the government said it would consider the latest scientific advice on masks and it seemed likely to encourage their use — a decision complicated by the shortage of masks for people who work in hospitals and nursing homes. Some of SAGE's internal debates play out in competing research studies published by their authors. A few days after Imperial College released its dire projections about the deadliness of the virus, a team at Oxford University published a study that considered a scenario in which more than half

of the population might already have been infected — a theory that, if valid, would argue for a less draconian response. Scientists, of course, often disagree and change their minds, based on new data. To some, that is yet another argument for lifting the veil on the advisory group.

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So why has the NYT reported this? Have they got it wrong? LONDON — As the British government comes under mounting criticism for its response to the coronavirus — one that has left Britain vying with Italy and Spain as the worst hit countries in Europe — Prime Minister Boris Johnson and his aides have defended themselves by saying they are “guided by the science.” The trouble is, nobody knows what the science is. The government’s influential Scientific Advisory Group for Emergencies — known by its soothing acronym, SAGE — operates as a virtual black box. Its list of members is secret, its meetings are closed, its recommendations are private and the minutes of its deliberations are published much later, if at all. Yet officials invoke SAGE’s name endlessly without ever explaining how it comes up with its advice — or even who these scientists are. That lack of transparency has become a point of contention, as officials struggle to explain why they waited until late March to shift from a laissez-faire approach to the virus to the stricter measures adopted by other European countries. Critics say the delay may have worsened a

death toll now surging past 20,000, and they fault the government for leaving people in the dark about why it first chose this riskier path. With all the secrecy, even some of Britain's top scientists say they don't know whether they can trust the government's approach. "Is the science being followed by the government on coronavirus?" said David King, a former chief scientific adviser to the government. "I don't know, because I don't know what the advice is, and there isn't the freedom for the scientists to tell the public what their advice is." Professor King, who counseled Prime Minister Tony Blair on the foot-and-mouth disease that infected British farm animals in 2001, said there was no justification for the government to withhold either the advisory group's membership or the minutes of its meetings. Doing so, he said, eroded public trust in the government, especially given the bewildering twist and turns in its response. It also raises questions about an academic group that ought to be a point of pride for Britain: the country's best scientific minds, in fields from epidemiology to behavioral science, assembled from cutting-edge labs at Cambridge, Oxford, Imperial College and the London School of Hygiene and Tropical Medicine. "The names are likely to come out at some stage," said David Lidington, who served as a deputy to Mr. Johnson's predecessor, Theresa May. He warned that the government's lack of disclosure would only cause more headaches later. "There is the risk that if names leak out

after a time it becomes a great shock-horror," he said, adding that it would be better to make a virtue of transparency. Even now, outside scientists and doctors are second-guessing the advice of these unnamed authorities. Why, for example, did SAGE recommend less stringent social-distancing measures on March 9, when France and Ireland were banning large events and ordering lockdowns, and there was ample evidence from Italy of the epidemic's rapid and lethal spread? (The unusual disclosure came in a report the government posted about the predicted effects of various social-distancing measures.) Why in late February did a subgroup of SAGE experts underestimate the percentage of people who would have to be hospitalized as a result of contracting the virus, and why did their models underestimate the speed at which the pathogen spread? Why did those scientists agree to classify the risk level of the contagion to the public as "moderate," even after weeks of evidence that it was being transmitted between humans in China? Why, after Imperial College London published a frightening study on March 16 that projected up to 500,000 deaths if Britain did not act more aggressively to curb the virus, did Mr. Johnson wait another full week to close nonessential shops and order people to stay in their homes? "Political decisions are often framed as following the best scientific advice," said Connor Rochford, a physician and former consultant at McKinsey & Company. "But science is nothing more than a normative

claim about how we ought to make a decision. These are best-guess estimates." Some said the frequent references of Mr. Johnson and his aides to the scientists should be a warning sign. If, as is likely, the government's handling of the crisis is scrutinized in a future parliamentary inquiry, officials are likely to justify their actions by saying they were listening to the experts. "It has become a shield for them," said Devi Sridhar, director of the global health governance program at Edinburgh University. "If things go off, you can always say, 'Well, it was the experts who told us.'" The government has deflected pressure to identify the group's members or how many there are by noting that Patrick Vallance, the current chief scientific adviser, who chairs the group, regularly appears in public at news conferences. The government also posts brief reports from some of SAGE's subgroups, and the data that go into its models, on the internet. In a recent letter to Parliament, Professor Vallance said anonymity protected the security of scientists and also shielded them "from lobbying and other forms of unwanted influence which may hinder their ability to give impartial advice." He added that people were free to disclose their membership. One member who has — Jeremy Farrar, an infectious disease specialist who is the director of Wellcome Trust — acknowledged the limitations of the system when he recently told the BBCthat the New and Emerging Emerging Respiratory Virus Threats Advisory Group, which advises SAGE, underestimated the threat of

the contagion in March. “The U.K.,” Dr. Farrar added, “is likely to be certainly one of the worst, if not the worst, affected countries in Europe.” Another member who has become a household name, and a source of scrutiny for his eye-watering statements, is Neil Ferguson, an epidemiologist at Imperial College London. His team of modelers produced the March 16 report that prompted Downing Street to impose a lockdown (it was also influential at the White House, which embraced social distancing). Professor Ferguson, who collaborates with the World Health Organization and has advised other countries on how to deal with epidemics, later came down with symptoms of the virus himself. In late March, testifying before Parliament from self-isolation in his house, he generated more headlines when he said that Britain could keep its death toll under 20,000 if it stuck with strict social distancing. Professor Ferguson did not reply to requests to discuss his advice to the government or the deliberations of SAGE. But in an interview with The New York Times the day the March 16 report was published, he laid out the choice Britain faced: Manage the spread of the virus in a way that minimized deaths but allowed a significant percentage of the population to become infected — a situation known as “herd immunity.” Or tamp down transmission of the virus by imposing a lockdown of the kind the Chinese government did in Wuhan. In the end, he said, there was no choice but to take the latter course. “The U.K. has struggled in the

past few weeks in thinking about how to handle this outbreak long term," Professor Ferguson said. "We don't have a clear exit strategy, but we're going to have to suppress this virus, frankly indefinitely, until we have a vaccine. It's a difficult position for the world to be in." Until mid-March, Professor Ferguson, Professor Vallance and other scientists had appeared receptive to the case for "herd immunity." Then, confronted with new numbers that projected hospitals would be overwhelmed with patients and that the death toll would skyrocket, they pivoted to a suppression strategy.

https://www.msn.com/en-gb/news/coronavirus/the-secretive-group-guiding-the-uk-on-coronavirus/ar-BB137GAL
https://www.readytogo.net/smb/threads/why-is-the-government-keeping-the-science-secret.1514746/

www.whale.to/c/viral_mania_a.html
www.whale.to/a/infectious_scares.html
www.whale.to/a/roberts_b.html
www.whale.to/a/engelbrechtH.html
www.virusmyth.com/aids/

www.uofmhealth.org/health-library/hw235580
www.sciencedirect.com/topics/medicine-and-dentistry/vir

us-identification www.labome.com/method/Virus-Identification-and-Quantification.html

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