COVID-19 pandemics? The tests

by Fabio Franchi^a (Version 1, 3 April 2020,)

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Preface

The urgency to face interstitial pneumonia of initial unknown origin with high death rates and rapid spread¹, ² justified a hasty and gross operational approach. New operational hypothesis were put forth without being followed by an immediate thorough analysis and a correct methodologic approach.

It's time to re-assess the most crucial passages, particularly a few aspects of the definition, viral isolation and demonstration of cause and effect of the Coronavirus with respect to the respiratory syndrome.

Only then will the mysteries of COVID-19 (or SARS-CoV-2) be clarified³.

Definition of "case"

The one defined by the WHO includes three possibilities: "suspect case" ⁴, "probable" and "confirmed" ⁵.

As for the first definition, it includes a set of symptoms, signs, lab and radiological alterations within an epidemiologic context, while for the second one a diagnosis

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is allowed to be made (even though just a probable one) even by a gene amplification test with an inconcluding result (that means negative for one of the two proteins tested for and characteristic of the COVID), or when a generic test for all coronavirus is positive. Therefore, it can also include individuals with a flue syndrome due to common circulating coronaviruses.

In China for instance, out of 76.314 cases reported in an extensive review ⁶, 22, 4% were classified as "suspect cases", 14,6% as "clinically diagnosed" and 1,2% as "asymptomatic". This means that 37% of Chinese statistics reported cases up to that point had been diagnosed only on a clinical basis ("suspect cases" according to the WHO's definition). Yet mainstream information given to the population around the world presented them all as confirmed cases.

Always according to the WHO's definition, a "confirmed case" is based on a positive test result, of the only one test that is RT-PCR, and regardless of the symptoms. Therefore even those who are well and healthy will be considered in every respect a "case", even without any disorder, neither any lab's alteration (if not that of the infamous swab) nor radiological. The antibody research, which should have been considered fundamental in confirming or not an acute infection, has been neglected. It was available since the start, yet up until now it has not been used (because of the discordance with the RT-PCR test's results?). Other exams are mentioned, but they are not indispensable (always according to the WHO's definition) and in actual facts most times they have not been and are not used. It ensues, that deaths are considered as due to Coronavirus if such test results positive, even though the death's cause assessment must follow other rules (it must be identified the most important pathology which led to the exitus, and mentioned apart any other collateral or concurrent pathologies). By definition asymptomatic cases are included and, always by definition, if these die by any other reason, the established cause remains COVID-19. Always referring to the "cause of death" in Italy the criterion of "all in" was followed, while elsewhere, as in Germany, the more rational approach of recording the real cause was adopted (at least until mid-March 2020). This is partly where the huge difference in lethality lies between Italy and Germany (11,40% versus 0,9%, end of March 2020) and probably other countries (Austria, Norway, Czechia, Australia, Taiwan, Croatia, Philippines, Finland, Thailand: temporary lethality under 1%, as of 28th March 2020). The Head of the Italian Civil Protection Angelo Borrelli, during the 12 March Press Conference clearly stated that deaths involve people who died with Covid-19 (positive PCR test), without differentiating those who died because of Covid-19.

Because of the in excess statistics' alteration, the result is that the perception of danger is heightened accordingly, just considering this one factor.

Final remarks on the modalities of diagnosis: In Italy the one of "confirmed case" is made regardless of the symptoms and linked only to the test's result (RT-PCR nasopharyngeal swab). The diagnosis of "probable" and "suspect" case are made without performing the test or with inconclusive results to the test. In case of co-infection with "other pathogens" [i.e. virus and bacteria], Covid-19 gets a sort of pre-emptive right⁷. The WHO definition clearly states it ⁸.

RT-PCR TEST

How does it work?

It enables the search for the virus' nucleotide sequence.

A sample is taken from a patient. Then, in a lab, the nucleotide's sequence of the virus (if there is any), is extracted and copied many times, making large quantitites become minute so as to be determined through other methods.

As *Corman et al*, among the first ones to prepare the test, then extensively adopted, explain in greater detail: "our intention was to develop and provide a robust diagnostic methodology without having the viral material at disposal" ⁹. Nota Bene: "without having the viral material at disposal"! They obtained the genetic sequence via internet and that is what they worked on.

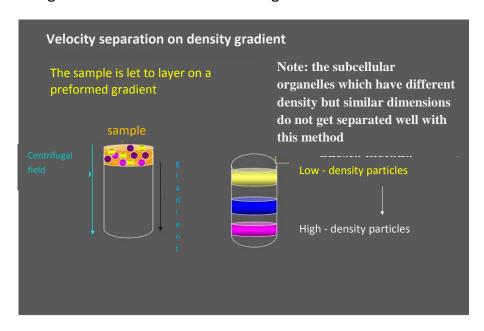
It is a crucial issue: before validating a test, it should be compared against the *gold standard*, that means against the virus whose presence it is tasked to reveal. Gene amplification does not replace this step. It is a very potent means, able to find minute quantities of genetic material multiplying it by two, manifold. With one cycle, from one fragment two are formed, from 2 to 4, from 4 to 8 ... With 20 consecutive cycles we obtain already one million copies. In short, it turns a needle in a haystack in a big stack of needles, well visible and examinable. Such test, even with COVID-19, does not amplify the whole virus, but it multiplies a small genetic sequence considered peculiar to that virus, and nothing else. How can it exactly be identified? It is absolutely indispensable that such small sequence reliably reveal that it is really that particular virus. But first it must be isolated.

The test (the swab taken from nasopharyngeal secretions which then undergoes gene amplification) has not been validated ¹⁰, ¹¹, it is not standardized ¹², it seems to be giving numerous false positives and false negatives ¹³, ¹⁴, ¹⁵.

Viral isolation

Isolation must be the first step, and it consists in the separation of the supposed virus from any other material (from latin *insulatum*). There is a precise procedure to be followed: the separation through ultracentrifugation in saccharose gradient. In short: from a supposedly infected cell culture the supernatant must be taken and centrifuged with such modalities. From there the material which sedimented in a layer corresponding to a particular density is taken, the viral one, specifically. A sample taken from that layer is then fixed and colored negative on a special support to be examined with an electronic microscope. Then it is photographed. The same operation must be carried out with precisely equal material, but not infected one (negative control). For a more technical description cfr. reference work by X-Y Ge et al. ¹⁶, as well as J Leibowitz et al ¹⁷.

Ultracentrifugation Procedure in saccharose gradient:



Ultracentrifugation gives optimal results in the following applications:

- Proteins, oligomeres and proteic complex purification
- Lipoproteins isolation
- Purification and separation of virus and viral particles
- () Isolation and separation of subcellular fractions
- Preparation of macromolecules through centrifugation in density gradient
- Nucleic acids purification (DNA) (RNA)
- Purification of extracellular vescicules (exosomes)
- → Nanoparticles separation

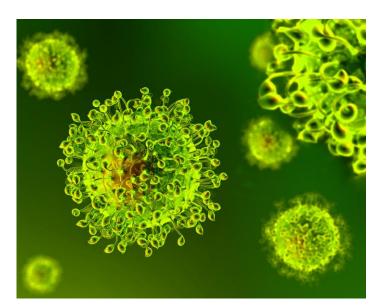
If, in this way, particles of the dimensions of a virus can be identified, all identical ones, while they are absent in the control, then they are analyzed (for constitutional proteins, genetic material), even by comparison against known viruses. In the same way reagents used for the tests can be derived (genetic sequencing, identification and production of specific antigens and then research and production of antibodies).

All the above to point out that the putative viral cause must FIRST be isolated (many elements, all identical, seen and photographed) THEN analyzed. This is basic logics.

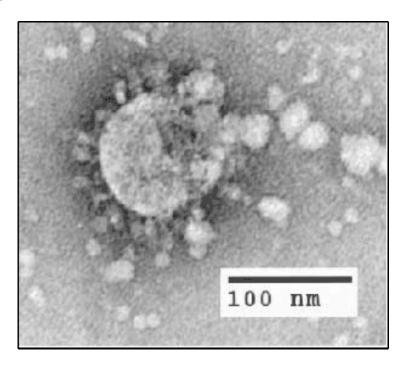
What is surprising is that with COVID-19 the first part of such procedure is missing. There are no pictures of the isolated virus in the published COVID-19 literature, if not of single elements out of context. You can find photos of ultrathin sections of tissues where you can see small circle agglomerations which are pointed at with arrows and called viral particles. Supposing they were such, they constitute less than 10% of the cell material which surrounds them. This is not actual, proper isolation. And there is more: there are serious doubts that those small circles are Coronaviridae. In fact, their size is smaller: their diameter (circa 65-70 nm) is inferior to the minimum one for Coronavirus (120-160 nm) ¹⁸. In actual facts other authors ¹⁹ report different diameters (80-220 nm), or 100-160nm²⁰, however these ones are totally out of range. Moreover, viruses are characteristically constituted by a few fundamental elements able to replicate identical copies of themselves. In short, biology does not provide for virus cubs!

If the diameter of small spheres is inferior to 30%, in volume they are even more so, that is they reduce to 1/3 circa. But this is not possible to maintain: it would inevitably mean a different composition and structure incompatible with beings endowed with the same nucleotidic sequence (since they belong to the same species).

For the discussion, we shall present one of the many fantasy computerized reconstructions which reinforces what we claim: it reveals everyone's conviction, experts and non-experts, that the COVID-19 virus is constituted by particles which are all identical.



Now, let us examine for comparison the Coronavirus deemed responsible for SARS (epidemic disease appeared in the Far East in 2002 and disappeared in 2004, for which a Coronavirus had been indicted, cfr. Wikipedia ²¹): the size and appearance correspond to the description. The diameter, spikes not included, is 100 nm circa.



A bat's Coronavirus, similar to the human SARS's one (disease appeared in 2003 and disappeared in 2004), was "isolated" and so presented on *Nature* Magazine²². Its appearance is compatible with the description even though its size is slightly bigger:

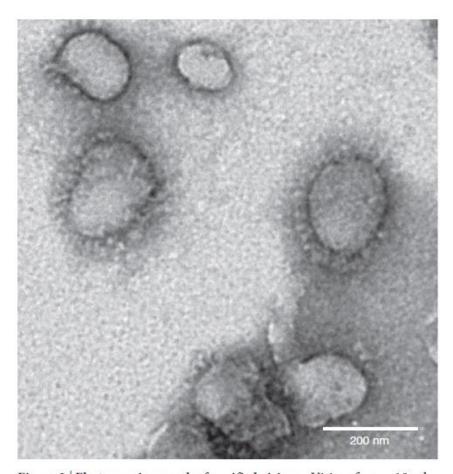
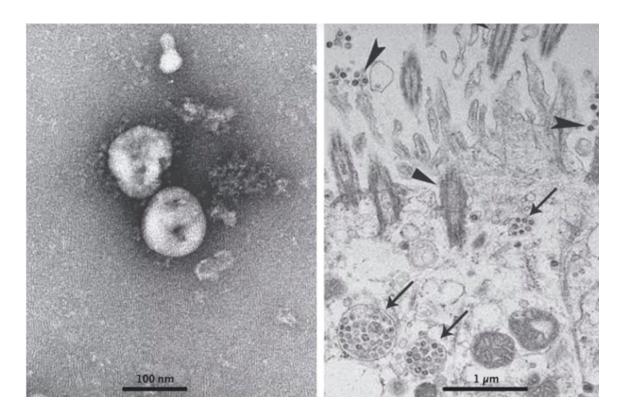


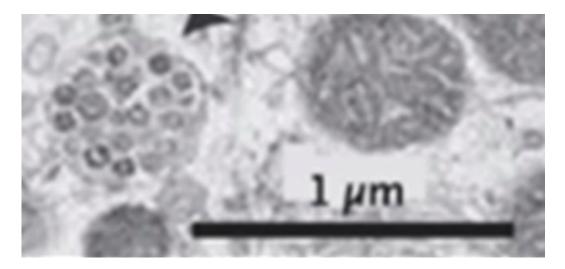
Figure 2 | Electron micrograph of purified virions. Virions from a 10-ml culture were collected, fixed and concentrated/purified by sucrose gradient centrifugation. The pelleted viral particles were suspended in 100 μl PBS,

COVID-19, whose photo was published on N Eng J Med in the current year ²³, instead has both different size and appearance:



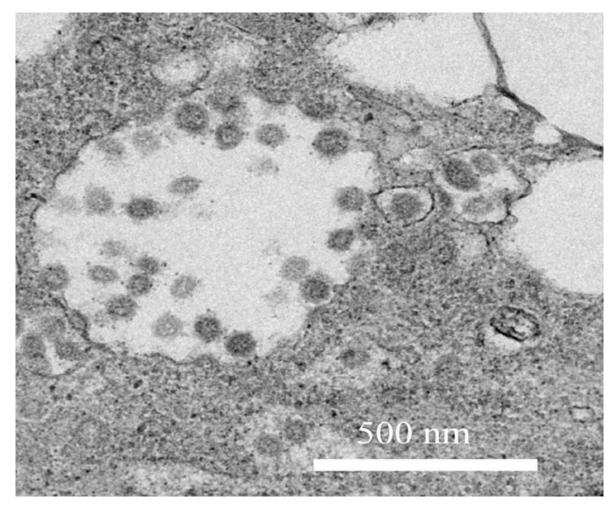
The two elements in the left photo correspond in their diameters to those of an ipothetical Coronavirus (around 100 nm, spikes excluded, which are not clearly visible). Viceversa the small particles divided in groups on the right are apparently too small to be Covid-19. It is remarkable that the round formations in the right photo do not have the same size of those in the left photo even though they are declared to be part of the same COVID-19 of the same study!

The image below is a blow up of the lower part in the right photo to allow for a better measurement.



As you can see by checking with a measurement software on the screen²⁴, the particles have a 65-75 nm diameter circa, except for one which has a 100 nm one.

The same can be observed in the photo published on Nature ²⁵ (size of the "viral particles": 67 nm average diameter, range 48-90):



The above photos are with every probability the best ones they were able to take.

Therefore, the doubt on the origin of such photographic materials gets all the more reinforced.

If the virus has not been isolated, and the test has been prepared without isolation, then the same reagents utilized (antigens and RNA strands) could have another origin. In the best of possible cases, that is that the particles in the photo are "COVID-19", then the material from which they derive the test is constituted for over 90% of cellular material. In short, if this is the case, there is no guarantee that the test is reliable and has the meaning it was given.

The above stated means that the demonstration process is weak and unreliable. On the other hand, many others realized the haste in implementing the procedure ²⁶, ²⁷, and reported the discrepancy of the results with the clinical results ²⁸. The excellent sensitivity and specificity claimed by the test makers ²⁹ clash with the much weaker ones found "on the ground". It is a practical side, much more important than the theoretical one.

In other words, there is no reason why the virus declared to be present in great quantity in cell cultures cannot be seen and photographed in the form of a carpet of viral particles, all identical, after ultracentrifugation. For a matter of such global importance it is legitimate to demand maximum reliability.

Possible meanings of the test

RT-PCR for COVID-19 has not been validated ³⁰, ³¹, it has not been standardized ³², it appears to be giving numerous false positives and false negatives ³³, ³⁴, ³⁵.

Therefore to test positive could mean: 1) the erratic result of a non-validated test; 2) cross reactivity; 3) the presence of a new transient virus, be it harmless or opportunistic; 3) the presence of another virus or pathogenic germ. To test negative can happen to individuals with all the clinical and epidemiological characteristics to be considered infected. Therefore, tests have been repeated even 6 times before obtaining the "desired" result³⁶, which is what seems to have happened even in the case of the hero doctor in Wuhan, Li Wenliang ³⁷.

How the test was prepared (RT-PCR)

The procedure which was adopted (and described in a very simplified way) is the following: the liquid for bronchoalveolar lavage in the first patients with interstitial bilateral pneumonia was put in a certainly non-infected cell culture. After a few days, with the onset of areas in cytolysis, the supernatant liquid was submitted to ultracentrifugation to eliminate cell residues on one hand, and extract presumably foreign nucleic acids. The latter have been amplified in various ways (even by RT-PCR, where RT means reverse transcriptase). Subsequently they were compared with known viral and bacterial sequences. An homology between some sequences and known Coronavirus ones was found. Subsequent passages led to find the complete nucleotide sequence of the new COVID-19 (single positive helix RNA of circa 30.000 bases). Slightly different nucleotide sequences have been identified by numerous research groups. A few small nucleotide sequences have then been isolated (100-200 nucleotides) characteristic of all Coronaviridae and some peculiar to COVID-19. Besides, ultrafine sections of presumably infected cell cultures have been checked under the electronic microscope (in the study by Zhu et al. 38), where they found the viral like particles visible in the above images. In the study by Zhou ³⁹ the procedure followed was the same. In both, therefore, the virus was not correctly isolated in the first place.

How to explain the epidemics

If the test is invalid, how can all that happened be explained? It can be explained with: a) a test epidemics (the more tests are performed, the more positive results to the test), b) a rise in winter mortality which hit like every year the weakest population (elderly and individuals with more underlying diseases), c) multiple factors non-infectious and infectious, including normally circulating coronaviruses. Such factors have been so far shamefully neglected ⁴⁰. A biased presentation of statistics can have had a role in this, coupled with a less than optimal medical approach⁴¹. Fear of a mortal disease has undoubtedly played a big part both for those who have been hit by it and for medical operators.

Demonstration of cause and effect

Even if we wanted to maintain – without solid evidence – that a new coronavirus spread first in China and then in Alzano Lombardo in Italy, the problems in the approach would not be finished. In actual facts, we would need the evidence demonstrating the causal link between virus and disease (viral bilateral interstitial pneumonia), which has not yet been provided (NEJM)⁴².

The above does not allow for denying that a new Coronavirus is circulating. Let us say it is, just for the purpose of understanding more deeply other issues that would arise that have not been addressed. Evidence based medicine (EBM) calls for it, and for good reason. Robert Koch had understood – circa 130 years ago – that the sole presence of a microorganism did not necessarily mean that it would cause some specific pathology, therefore he established a few logical criteria which are referred to still today (Koch's postulates ⁴³).

While waiting for such a study to be published, we can already observe that the disease (bilateral interstitial pneumonia) can manifest even without testing positive to COVID-19, and that the virus can be present in full health conditions, in the absence of disease or incubation in progress. In other words, COVID-19 (or its invalid test) is neither necessary nor sufficient to cause pneumonias or flu syndromes (in a conspicuous part of cases) within the current epidemics.

Bibliography

https://www.facebook.com/fabio.franchi.2.0/posts/139500407639452

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WHO: definition of case for surveillance purposes 18/02/2020

Suspect case requiring diagnostic tests (not to be reported at European level)
Patients with acute respiratory infection (sudden onset of at least one of the
following signs: cough, soar throat, shortness of breath requiring hospitalization
or not.

MOREOVER

In the 14 days prior to the onset of symptoms at least one of the following epidemiological criteria was met:

was in close contact with a confirmed or probable case of infection from SARS-CoV-2;

OR

Had a history of travel through areas with presumed ongoing spread in the community;

OR

had worked or works in a hospital treating patients with infection from SARS-CoV-2.

WHO: definition of case for surveillance purposes 18/02/2020

Probable case

A suspect case for whom testing for the SARS-CoV-2 is inconclusive (the result of the test from the laboratory) or who tested positive on a pancoronavirus test.

OR

Confirmed case

A person with laboratory confirmation of SARS-CoV-2 infection, irrespective of clinical signs and symptoms.

5

¹ Wuhan Municipal Health Commission. Press statement related to novel coronavirus infection (in Chinese) http://wiw.wuhan.gov.cn/front/web/showDetail/2020012709194 (2020).

² Zhou P et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270-3.

Amongst the mysteries: how is it possible that with the coming and going of Chinese people all over Europe, the first 2 outbreaks happened in Germany, and in Italy only Vò? Who would patient 1 (a 38 yr old marathon runner who had just one suspect contact: a Chinese person who resulted non infected) have gotten the disease from? More examples in this post:

for community-acquired pneumonia. Additional testing should not delay testing for COVID-19. As co-infections can occur, all patients that meet the suspected case definition should be tested for COVID-19 virus regardless of whether another respiratory pathogen is found.

The international definition of case envisages that a confirmed case is that of an individual with a laboratory confirmation of the virus causing COVID19 regardless of clinical signs and symptoms

https://www.ecdc.europa.eu/ en/case-definition-andeuropean-surveillance-human-infection-novel-coronavirus-2019-ncov

⁹ Corman V M et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):pii=2000045.

The epidemics currently underway of the novel coronavirus (2019-nCoV) recently found represents a challenge for the public health laboratories since isolated virus samples are not available while there is growing evidence that the epidemics is more widespread than initially thought, and that there is already an international spread amongst travelers. Our goal: we aimed at developing and implementing a solid diagnostic methodology to be used in a public health laboratory environment without having any viral material.

¹⁰ Xiao S-Y. Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol. 2020;92:464–467.

"Another concern regarding the nucleic acid tests is that **there was not enough time to assess its sensitivity and specificity.** On the basis of personal communications with our colleagues, a significant part of the patients who otherwise are included in the diagnosis based on the clinical results and CT thorax, including many hospitalized patients resulted negative to the viral RNA. Other common respiratory etiologies such as influenza were excluded. These ones remain "suspect" cases and can reflect the **false negativity** in the sampling. In some patients, the virus can be present in the lower respiratory secretion but absent in the upper respiratory tract. **With the current test, it is therefore difficult to obtain a significant assessment of the percentage of infected asymptomatic patients."**

⁶ The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. The Epidemiological Characteristics of an Outbreak of 2019 Novel Coronavirus Diseases (COVID-19) — China, 2020. Chinese Center for Disease Control and Prevention CCDC Weekly.2020;2(8): 113-122.

⁷ WHO Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance 19 March 2020.

¹¹ James Gallagher. Are coronavirus tests flawed? 13 February 2020 https://www.bbc.com/news/health-51491763

MAcgp2Q8J:https://www.cebm.net/2020/03/is-there-any-significant-difference-in-sensitivity-of-covid-19-virus-sars-cov-2-tests-based-on-swabs-from-oropharyngeal-op-vs-nasopharyngeal-np-sampling-vs-both/+&cd=4&hl=it&ct=clnk&gl=it

VERDICT

The only current COVID-19 specific data comparing OP with NP comes from two low quality, non-peer-reviewed studies and should be viewed with caution. It is not possible to accurately assess sensitivity from the existing data and there are no data to assess the diagnostic impact of combining both tests.

DOI: 10.1056/NEJMoa2001017.

¹² Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. February 26, 2020; 1-23. https://doi.org/10.1148/radiol.2020200642.

¹³ Catherine Carver, Nick Jones. Is there any significant difference in sensitivity of COVID-19 virus (SARS-CoV-2) tests based on swabs from oropharyngeal (OP) vs nasopharyngeal (NP) sampling vs both? CEBM research March 26, 2020 Centre for Evidence Based Medicine. 25th March 2020 https://webcache.googleusercontent.com/search?q=cache:RQ-

¹⁴ Tan Yucheng Who are false negative patients with new coronary pneumonia Southern People Weekly 9 febbraio https://mp.weixin.gg.com/s/RpXRE8Ow5nHeaLhxIEr-Ng

¹⁵ Xingzhi Xie et al. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing. Radiology Published Online:Feb 12 2020https://doi.org/10.1148/radiol.2020200343

¹⁶ Xing-Yi Ge et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 2013;503:535-8.

¹⁷ Leibowitz J et al. Coronaviruses: Propagation, Quantification, Storage, and Construction of Recombinant Mouse Hepatitis Virus. Curr Protoc Microbiol. 2011 May; CHAPTER: Unit–15E.1. doi:10.1002/9780471729259.mc15e01s21.

¹⁸ International Committee on Taxonomy of Viruses ICTV

https://talk.ictvonline.org/ictv-reports/ictv 9th report/positive-sense-rna-viruses-2011/w/posrna viruses/222/coronaviridae

¹⁹ Megan Culler Freeman and Mark R. Denison. Coronaviruses. Nelson's Textbook of Pediatrics, 20th Edition. **C**hapter 264: 1613-1616.e1.

²⁰ Raphael Dolin. Coronavirus infections, including SARS. Etiologic Agent, in Harrison's Principles of Internal Medicine, 17th Edition.

²¹ https://it.wikipedia.org/wiki/SARS-CoV

²² Xing-Yi Ge et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 2013;503:535-8.

 $^{^{23}}$ Zhu N et al. A novel Coronavirus from patients with Pneumonia in China, 2019. N Engl J Med 2020;382:727-33.

²⁴ J Ruler

²⁵ Zhou P et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270-3.

²⁶ Sheridan C. Coronavirus and the race to distribute reliable diagnostics. Nature. 19 FEBRUARY 2020. https://www.nature.com/articles/d41587-020-00002-2

Tan Wei, vicepresident of the professional Committee for medical radiology in the Hubei province, thinks that the results of the TC are quite consistent with the novel coronary pneumonia, but the negative detection of nucleic acid represents circa 30% -40%.

³⁰ Xiao S-Y. Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol. 2020;92:464–467.

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²⁷ Gallagher J. Are Coronavirus test flawed? BBC. February 13, 2020. https://www.bbc.com/news/health-51491763

²⁸ https://mp.weixin.gg.com/s/RpXRE8Ow5nHeaLhxIEr-Ng

²⁹ Corman (op cit)

³¹ James Gallagher. Are coronavirus tests flawed? 13 February 2020 https://www.bbc.com/news/health-51491763

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³⁴ Tan Yucheng. Who are the false negative patients with a coronary pneumonia Southern People Weekly 9 febbraio https://mp.weixin.qq.com/s/RpXRE8Ow5nHeaLhxIEr-Ng

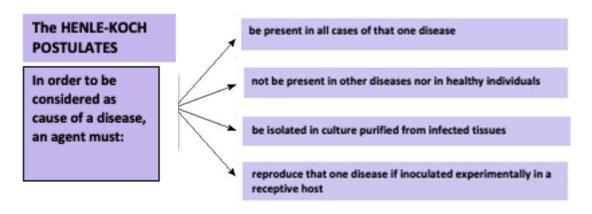
³⁵ Xingzhi Xie et al. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing. Radiology Published Online:Feb 12 2020https://doi.org/10.1148/radiol.2020200343

³⁶ Tan Yucheng (op cit).

³⁷ Gallagher, Are coronavirus tests flawed? 13 February 2020 https://www.bbc.com/news/health-51491763

[&]quot;... Dr Li Wenliang, who first raised concerns about the disease and has been hailed as a hero in China after dying from it. Dr Li posted a picture of himself on social media from his hospital bed, on 31 Jan. The next day, he said, he had been diagnosed for coronavirus. He said his test results had come back negative on multiple occasions before he had finally been diagnosed.

Postulates can be synthetisezed in 4 simple points:



³⁸ Zhu et al (op cit).

³⁹ Zhou et al. (op cit)

⁴⁰ **1) Previous Vaccinations**. Particularly in the worst hit area (Brescia Bergamo), the current epidemics has been preceded within a short time by an extensive vaccination campaign for the anti influenza, anti pneumococcus, anti-HZV, antimeningococcus. **2) On the severity of the disease,** a lot came from the stress on the health system which was unprepared thanks to the severe cuts it underwent over the past 10 years. **3) The workload and the emotional tension** of the medical staff has been excessive and prolonged. **4) Experimental drugs have been tested** which proved no effectiveness whatsoever and high toxicity, according to aggressive protocols, "justified" by the presence of a "lethal disease", dangerous mainly for those who were already ill, the elderly or those with concurrent diseases, cortisone has been used in a different way than it was prescribed for viral pneumonias.

⁴¹ Samuele Ceruti, Medicina d'Urgenza, Lugano, Svizzera. Lettera aperta ai colleghi italiani https://www.vglobale.it/wp-content/uploads/2020/03/Coronavirus-dati-aggiornati-al-12-marzo-2020.pdf

⁴² Zhu N et al. (op. cit).

[&]quot;Even though our study does not satisfy Koch's postulates, ...

⁴³ Postulates by Koch-Henle