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Response to Comments on Kämmerer, et al. (2023) regarding RT-PCR Testing

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Abstract

Franchi and Tomsic (2023) correctly note that our review (Kämmerer et al., 2023) "has a clear focus on the technical aspect of RT-PCR, which is only one piece of the COVID-19 puzzle" and they ask for a more comprehensive discussion beyond our focus on the laboratory assay. They point to the lack of a specific definition of COVID-19 disease and conclude that, in order to test the first and the second of Koch's postulates, there must be both a purified germ and a specified disease, neither of which was available for COVID-19. In reply, we address two questions they did not ask: 1. Are clinical symptoms induced by SARS-CoV-2 corroborated by RT-PCR? 2. Are Koch's postulates valid for viruses? We assert that testing asymptomatic people is useless, whereas testing patients with clinical symptoms for a respiratory disease may enable a physician to confirm or reject a suspected diagnosis. Determining a diagnosis for any given patient is the *physician's challenge*, while the *researcher* is responsible to show that the available tools are as near optimal as possible and to clarify the limitations of any such tools. Because there are no tools suitable for comprehensive and exclusive detection of infectious pathogens, we need to proceed carefully in applying the limited tools that do exist for tracing and tracking viral pathogens, to avoid under- or over-estimating a real or suspected pandemic.

Keywords: *Chamberland-Pasteur filter, clinical symptoms, COVID-19 disease, CT-scan, electron microscopy, discovery of viruses, Koch's postulates, porcelain filter, RT-PCR*

1. Are clinical symptoms induced by SARS-CoV-2 corroborated by RT-PCR?

Determining a patient's diagnosis is a challenging task in general, but especially so for SARS-CoV-2 which is a respiratory pathogen with symptoms extremely similar to those of other pathogens inducing respiratory infections, i.e., coughing, running nose, dyspnea, loss of smell/taste and fever (Czubak et al., 2021). The objection by Franchi and Tomsic makes sense, but with patients severely sick exhibiting dyspnea plus fever, diagnostic tools such as a CT scan accompanied by RT-qPCR and a viral culture could assist a physician in confirming or rejecting a particular diagnosis.

Of note, any laboratory assay requires intensive validation before routine application in a clinical setting. This was the reason for two of us (RL, PB) investing additional work in evaluating RT-qPCR for SARS-CoV-2 (Voogd et al., 2022) in order to validate a CRISPR Cas-based point-of-care test for the detection of SARS-CoV-2. It was demonstrated that, after RT-qPCR, it is of pivotal importance to run an agarose gel electrophoresis and to sequence the generated PCR amplicons. It was revealed that a positive RT-qPCR test still harbors the risk of picking up something other than SARS-CoV-2 alone. Samples from primary care patients suspicious for SARS-CoV-2, since the patients presented themselves with clinical symptoms of a respiratory infection, after RT-qPCR, were found to be positive for other viral and bacterial pathogens and even human genomic DNA (Voogd et al., 2022). Thus, solely trusting the outcome of a positive RT-qPCR test result risks a wrong diagnosis even with optimized commercial kits.

Viral culturing could provide a confirmation of the presence of infectious SARS-CoV-2 virus particles using a Ct-value cut-off of less than 24 for the E-gene (Bullard et al., 2020). When positive at this cut-off, it could be interesting to invest money in order to search for infectious viruses such as SARS-CoV-2 and by doing so to confirm a suspected diagnosis drawn by a physician. False negatives have been reported and have affected the hospitals with vulnerable patients during the pandemic (Pecoraro et al., 2022; Voogd et al., 2022), leading to hospital outbreaks and loss of life. Franchi et al., stated that this automatically means that other causes have to be sought. However, according to Voogd et al. (2022) their assertion is not always true, because SARS-CoV-2 may still be the causative agent — likewise symptoms could have been caused by influenza or other types of respiratory viruses.

1. Are Koch's postulates valid for viruses?

Due to their small size (15-400 nm; coronaviruses 80-140 nm), viruses cannot be visualized with a light microscope. They can be seen only with an electron microscope. That kind of instrument did not become available until 1931 and because of its cost is only accessible even today in fairly advanced and expensive laboratories. To be viewed under electron microscopes, a large number of viruses is required. To obtain them, viruses from patient samples are first propagated in a cell culture process referred to as "isolation" (as in the Killingley et al. experiment referred to below here).

A comprehensive review on the historicity of Koch's postulates is provided by Gradmann (2008). That author argues that the postulates were first formulated by one of Koch's students, namely Friedrich Löffler. During Koch's lifetime — born in 1843 and died in 1910 — the existence of viruses as disease agents capable of replication had to be assumed on the basis of indirect evidence. Using porcelain filters of the type Chamberland (1884) developed in Pasteur's laboratory, Ivanovsky (1892) was able to establish that a tobacco virus much smaller than a bacterium could replicate rapidly and infect tobacco leaves. A few years later in 1898, Friedrich Löffler and Paul Frosch using a similar filter made the case for foot and mouth disease in cattle, sheep, and horses. These experiments, particularly the latter one, not only met

the requirements of Koch's postulates but defined them (Horzinek, 1997). In the lattter case, the isolation of the viruses by filtration and the subsequent infection of previously healthy animals with the same disease, led Löffler to formulate what would become known as "Koch's postulates": (1) that a causative pathogen must be detectable in all patients with specific symptoms; (2) that the pathogen must be isolated and cultured in pure form; and (3) that the pathogen in the pure culture must have the power to produce the disease in healthy organisms.

It should also be mentioned that Koch's postulates originated when microbiology was taking its first baby steps. They were constructed decades before viruses would become detectable with the electronic microscope that had not yet been invented. They were written long before advanced cell cultures would become possible. Now, given more than 100 years of improved laboratory methods, the original postulates are neither useful for routine clinical diagnosis of disease, nor are they necessary due to state-of-the-art techniques such as DNA/RNA (Sanger/next generation) sequencing, Western blot analyses, protein sequencing, and proof of virus-specific antibodies in the serum of infected patients. With respect to SARS-CoV-2, the particular virus itself was reportedly isolated from patients via standard-cell culture procedures and was characterized by complete genome sequencing and antigen specification, as described in Jefferson et al. (2020), Lu et al. (2020), Ren et al. (2020), Zhu et al. (2020), and Jaafar et al. (2021). Furthermore, although successful challenge experiments in "naïve" organisms — ones with no pre-existing immunity or cross-immunity to the pathogen of interest which respond with the typical symptoms to controlled infection by a defined strain — are difficult to obtain in the human population, with respect to SARS-CoV-2, even that difficult confirmation of viral infection has been found in a human challenge experiment conducted at Royal Free London National Health Service Foundation Trust. They employed fully informed participants who were willing to take the risks entailed and achieved successful infection with SARS-CoV-2 in 52.9% (18) of the 34 young-adult volunteers between the ages of 18-29 (Killingley, 2022) who completed the exploratory challenge experiment.[1] Of those infected patients, 89% reported mild to moderate symptoms and 11% remained asymptomatic. The SARS-CoV-2 viral disease agent in our view exists.

All that being said, nevertheless, we believe there was a grossly negligent omission during the COVID-19 pandemic: regular and sufficient negative and positive controls did not exclude the co-presence of pathogen(s) other than SARS-CoV-2 (except in the just-mentioned Killingley experiment) which might be causing the observed COVID-19 disease symptoms. Additionally, the symptoms used in diagnosis are so general and common in respiratory diseases that it "… may not be possible to distinguish among the viral diseases under study judging only by the clinical presentation" (Czubak et al., 2021). Among the disease agents that cannot be definitively excluded are seasonal flu viruses which have been identified as co-infective by Wuhan researchers (Yue et al., 2020) in some persons diagnosed with SARS-CoV-2 infection. Given that there are no "virus-type-specific" therapies that distinguish all the types of respiratory viruses, molecular diagnostics hardly have anything more than mere academic value. The critical information guiding the choice of therapies would need to take account of co-infecting bacteria and fungi possibly accompanying any respiratory viruses and for which a specific therapy could have helped or even saved the lives of many victims — such as those "COVID-19 patients" who died with non-detected aspergillus and might have survived on anti-fungal therapy (Evert et al., 2021).

[1] Two of the original 36 volunteers who were inoculated with SARS-CoV-2 virus were excluded before completion of the experiment on account of having "seroconverted" between the time of initial screening and subsequent inoculation.

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