Issues with the RT-PCR Coronavirus Test

https://theinfectiousmyth.com/coronavirus/RT-PCR_Test_Issues.php

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Version 3

This is an analysis of the so-called coronavirus test, based on RT-PCR technology. It is based significantly on my recent reading of a 2017 article on potential problems with RT-PCR by Professor Stephen Bustin, a world expert, a podcast that I recently conducted with him and the MIQE guidelines for operating and reporting on RT-PCR data. This article does not question whether the RNA used in the test is viral or endogenous. If the RNA is not viral, then clearly the RT-PCR coronavirus test is of no value. This web page does not contain references, for those you should consult the fully referenced Coronavirus Panic Critique.

The PCR Cycle Number

The PCR algorithm is cyclical. At each cycle it generates approximately double the amount of DNA (which, in RT-PCR, corresponding to the RNA that the process started with). When used as a test you don't know the amount of starting material, but the amount of DNA at the end of each cycle will be shown indirectly by fluorescent molecules that are attached to the probes. The amount of light produced after every step will then approximately double, and when it reaches a certain intensity the process is halted and the sample is declared positive (implying infected). If, after a certain number of cycles, there is still not sufficient DNA, then the sample is declared negative (implying not infected). This cycle number (Ct) used to separate positive from negative is arbitrary, and is not the same for every organization doing testing. For example, there is a paper published that reported using 36 as the cutoff for positive, 37-39 as indeterminate, requiring more testing, and above 39 as negative. Another paper used 37 as the cutoff, with no intermediate zone. In a list of test kits approved by the US FDA one manufacturer each recommended 30 cycles, 31, 35, 36, 37, 38 and 39. 40 cycles was most popular, chosen by 12 manufacturers, and one each recommended 43 and 45.

Meaning of the Ct

Implicit in using a Ct number is the assumption that approximately the same amount of original RNA (within a multiple of two) will produce the same Ct number. However, there are many possibilities for error in RT-PCR. There are inefficiencies in extracting the RNA, even larger inefficiencies in converting the RNA to complementary DNA (Bustin noted that efficiency is rarely over 50% and can easily vary by a factor of 10), and inefficiencies in the PCR process itself. Bustin, in the podcast, described reliance on an arbitrary Ct number as "absolute nonsense, it makes no sense whatsoever". It certainly cannot be assumed that the same Ct number on tests done at different laboratories indicates the same original quantity of RNA.

Limits on Cycles

Professor Bustin stated that cycling more than 35 times was unwise, but it appears that nobody is limiting cycles to 35 or less (the MIQE guidelines recommend less than 40). Cycling too much could result in false positives as background fluorescence builds up in the PCR reaction.

Ct and Number of Positive Tests

The Ct cycle number will significantly influence the number of positive tests. If the Ct was

changed from 37 to 35 there would be fewer positive tests, and if changed to 39 there would more positive tests. Even if the Ct number was standardized, it would still have different meaning depending on the specific machines, chemicals and procedures used by different labs, and even within the same lab changes could still be found between different runs of samples. Without simultaneously amplifying a known quantity of 'spiked' RNA, it cannot be assumed that with consistent Ct numbers can be used to consistently provide a boundary between positive and negative.

Is the Amount Meaningful?

If the process is efficient, a large number of cycles could detect as little as three molecules of RNA. If there are people who had such a small amount of virus in their body, causing no health problems, they would still test positive.

Is the Virus Functional?

If there are only parts of viruses present, or defective virus particles, that are not infectious, they would still produce positive tests. The tests do not prove that pathogenic, replicable virus is present.

Can RT-PCR Distinguish Infected from Uninfected

No.

How RT-PCR Works in More Detail

The following steps are used to test for particular RNA:

1. RNA must be extracted from a sample. This must be done carefully to ensure that DNA is eliminated, and that chemicals that might inhibit further steps are not included. It is impossible to ensure absolute purity of the RNA.
2. RNA must be converted to complementary DNA (cDNA). This uses the enzyme Reverse Transcriptase and is never terribly efficient (50%). The amount of DNA produced can vary significantly, depending on numerous factors, perhaps by a factor of 10 (it used to be a factor of 100).
3. In the PCR part of the process, cDNA is present with primers and a probe (and possibly some stray DNA from the sample). The primers delimit the beginning and the ending of the cDNA that is intended to be duplicated. The probe helps ensure that RNA is only duplicated if it matches the primers (which are quite short) and the probe. At each cycle of this process (PCR proper) the amount of DNA is approximately doubled. Fluorescent markers are attached to the probe so that, at each step, the amount of light can be used to estimate how much DNA has been generated.
4. Optionally, the resulting DNA can be sequenced to determine exactly what the bases ('string of four different DNA beads') are.

Errors and inefficiencies can occur at every step. It is not possible to actually estimate quantities unless the reaction is 'spiked' with a known amount of a different RNA, which is also duplicated. Then the PCR cycle number can be roughly correlated with the original quantity of material.

Is There Proof There Are Problems, Or Is This Just a Hypothesis?

There are now several papers that illustrate essentially impossible testing results. A paper from

China reported on consecutive testing results, defined as either Negative (N), Positive (P) or Dubious (D, presumably intermediate). Results for 29 people with inexplicable results out of about 600 patients were: 1 DDPDD 2 NNPN 3 NNNPN 4 DNPN 5 NNDP 6 NDP 7 DNP 8 NDDPN 9 NNNDPN 10 NNPD 11 DNP 12 NNNP 13 PPNDPN 14 PNPPP 15 DPNPNN 16 PNNP 17 NPNPN 18 PNP 19 NPNP 20 PNPN 21 PNP 22 PNP 23 PNP 24 PNDDP 25 PNPNN 26 PNPP 27 PNP 28 PNPN 29 PNP. A study from Singapore did tests almost daily on 18 patients and the majority went from Positive to Negative back to Positive at least once, and up to four times in one patient. In China they have found that 5-14% of patients who have been cleared, with two consecutive negative tests, have later tested positive again, usually without new symptoms. In South Korea they recently reported 91 such patients. A 68 year old Chinese man went to hospital with symptoms, and tested positive. After his symptoms resolved and he tested negative twice he was released. But he tested positive again, and was readmitted, was released again, tested positive again, was readmitted, and then was released for a third time.

Conclusions

RT-PCR testing for the Coronavirus seems to be designed to produce as many positive tests as possible. The fear of missing a true positive is so great that those designing the specific testing methodology based on RT-PCR completely ignore the risk of false positives. False positives make the epidemic appear larger, and justify the complete shutdown of the economy, locking people in their own homes, and destroying just about everything in the lives of people that brings them joy, such as playing ball in the park, going for coffee with a friend, going to the theater or a sports event, going swimming, going to the county fair.