# The Incredible and Scary Truth about COVID-19 Tests

David Crowe
David.Crowe@theinfectiousmyth.com
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A lot depends on the result of your COVID-19 test, whether it is positive, indicating infection or, big sigh of relief, negative, indicating that you are not infected. But is there such a thing as "the" COVID-19 test? Indeed there is not. There are many and each is looking for different things and making different decisions about whether those things are present or not.

## The Test is Not Binary

It is important to understand that the COVID-19 test does not inherently have only two values. The test uses multiple cycles of the PCR (Polymerase Chain Reaction) technology, with an arbitrary count of cycles being the boundary between positive and negative, usually interpreted as infected and uninfected. Not only is this division arbitrary, but we know that it does not work that well because there are numerous published examples of people testing positive, then negative, then positive again, within a few days. There is, so far, no explanation of this phenomenon amongst people who are unwilling to question the test technology, test implementation or viral theory, although manufacturers do obliquely refer to this problem in their technical documentation by admitting that false positives can be caused by "non-specific signals in the assay" or, more directly, "As with other tests, false-positive results may occur."

Imagine a game dreamed up by Harry Potter and Lewis Carroll. It is played in a field and the bounds are a circle that is not marked. If someone yells "out of bounds" the referee goes to the centre with a curled-up flamingo and rotates it a number of times, a number chosen arbitrarily by the referee. Some choose 30, and some choose other numbers up to 45. Additionally, different referees have flamingoes of different sizes, and sometimes they are curled up more tightly than at other times. But, if you are within the, say, 37 flamingo turns, you are safe, and if not, out of bounds. Welcome to the world of testing for the coronavirus.

## Complexity

Coronavirus tests are performed by sophisticated machines with simple interfaces. Program the parameters of the test, pop in the samples, and in a relatively short time, the results are displayed, sometimes as a graph, or other times as simply as "Positive", "Negative" or "Invalid". But the process is not simple. First the RNA needs to be extracted from the sample, which will include a lot coming from your cells, from bacteria, or other sources, as well as possibly some from viral particles, all of which could possibly react with a later stage, causing a false positive. It is also important at this step to eliminate non-RNA substances that could interfere with following steps.

Secondly, the RNA needs to be converted into DNA, because PCR only works with DNA. This process uses the enzyme Reverse Transcriptase, hence the moniker RT-PCR for the combination of RNA conversion followed by standard PCR. The RNA to complementary DNA (cDNA) conversion process is quite inefficient. Stephen Bustin, a professor at Anglia Ruskin university, and perhaps the world's leading expert on quality control of RT-PCR, told me in a recent interview (infectiousmyth.podbean.com/e/the-infectious-myth-stephen-bustin-on-challenges-with-rt-pcr) that the amount of DNA obtained can vary widely, easily by a factor of 10. Since the PCR cycle number is a measure of the amount of material obtained, different efficiencies at the RT step essentially invalidate the simple use of the PCR cycle number. Two different test setups in two different labs, that both use the PCR cycle number 35 as a cutoff, may actually have the cutoff between negative and positive at wildly different places.

Finally, the third step, pure PCR occurs. As described above, this is repeated many times. On each cycle the DNA is unrolled from the double helix into two strands, the portion of interest is duplicated, and the DNA rolls up again.

You may think this explanation is complicated. Yes. It is a complicated process. And although a fancy machine makes it simple to operate, it doesn't mean that every machine, every lab and every operator gets comparable results. Your situation is even worse than the operators because you will likely just be told either "Infected" or "Clear".

## A Potpourri of Tests

The NHS does not exert much control over the choice of COVID-19 test, allowing in-house validation of test kits (http://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/03/guidance-and-sop-covid-19-virus-testing-in-nhs-laboratories-v1.pdf) although, more recently, it started to insist that commercially available, rather than in-house tests be used (www.telegraph.co.uk/news/2020/04/21/public-health-england-admits-coronavirus-tests-used-send-nhs). The US Food and Drug Administration, on the other hand, requires at least a façade of test approval through their Emergency Use Authorizations. I downloaded 33 of the test kit instructions, hopefully a representative sample, to try to see how the tests differed in what they were looking for, how long they were looking, and how they decided whether they had found it or not. I

also scanned the test limitations, to see whether the manufacturers thought their tests were perfect or not. If you are a true masochist, you can check my analysis at:

https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations

## The Number of Flamingo Turns

For some tests in the FDA list, the number of PCR cycles to distinguish positive from negative is not specified, but for most, it is. In general, the more PCR cycles, the more likely that a false positive will be obtained, and the fewer cycles, the more likely a false negative will be obtained. One manufacturer each recommended 30 cycles, 31, 35, 36, 37, 38 and 39. 40 cycles was most popular, chosen by 12 manufacturers, and two recommended 43 and 45. The MIQE (Minimum Information for Publication of Quantitative RT-PCR Experiments) guidelines for operation and reporting of RT-PCR states that the use of 40 or more cycles is unwise (academic.oup.com/clinchem/article/55/4/611/5631762). Bustin's advice in my interview with him was that not more than 35 cycles be used. With either 35 or less than 40, the majority of COVID-19 RT-PCR tests approved by the FDA may be pushing RT-PCR to its limits or beyond.

## What is Being Looked For?

The RT-PCR tests look for only a tiny fraction of the COVID-19 genome. And different tests look for different tiny fractions. Most do not specify the size of the portions, but a test developed by Charité Berlin (not on the FDA list) looks for the RdRp and E genes, which amount to 213 bases out of about 30,000 for the entire genome, or less than one percent. On the FDA list, the tests reference the E, N and S genes and portions of the ORF (Open Reading Frame). What is most important to know is not what the function of these RNA segments is, but simply that the tests are looking for very different things. It is as if we went looking for leopards with one person using spots as the guide, another the claws, another the teeth and another the eyes.

Worse than differences in what they are looking for is the way of defining whether they have found it. Some tests look for one portion that must be present for the test to be declared positive. Others look for two portions and both must be positive, while others only require one of the two to be positive. Some tests look for three portions, and generally only require two to be detected, although one test requires all three.

This is worth thinking about. A test that looks for three portions of the genome is generally happy if two are found. That means that we can have a leopard without spots as long as it has leopard-like claws and teeth. Or spots and teeth, but different claws. What does it mean to have a genome of a very simple creature like a virus, for which any part can be missing, but we still say it is what we are looking for? And if we only have 1% of an animal, is it possible we will decide it is a leopard when it is actually an ocelot?

## Limitations of the Test

Each test comes with a list of limitations. And the majority probably apply to all tests, even though they are only listed in some. These include noting that the test is only looking for RNA, and does not prove that a virus is present, and certainly cannot prove that the virus is functional. Some note that RNA from the virus may persist after the infection has been resolved.

A variety of reasons for false negatives and false positives are given. While public health agencies are generally not interested in false positives, this problem has the power to magnify the epidemic, as well as turning people's lives upside down. Some tests note correctly that false positives increase as the number of actual infections in the population being tested decrease. Also, RT-PCR is so ultra-sensitive, that a tiny amount of contamination at any stage of the process can result in a false positive, and the manufacturers warn about this. Some tests indicate that other coronaviruses may cause positive test results, but many coronaviruses are not believed to be very pathogenic, so this is equivalent to a false positive to the person receiving the misleading result. A mix-up of two specimens may cause one false positive and one false negative, as people are given the wrong results.

Some tests indicate correctly that the presence of the coronavirus RNA, even if taken as proof of viral infection, does not prove that it is the cause of any symptoms being experienced.

Many also recommend that the test alone not be used to make a diagnosis but that clinical information (such as symptoms) and a doctor's opinion be incorporated.

Many tests admit they have not been tested on immunocompromised people or on people with symptoms, indicating that the manufacturers are concerned about the accuracy in these groups.

## Impact on Your Life

One story from China illustrates the absurdity of the current situation with COVID-19 testing, the impact on people's lives, and the unwillingness of medical professionals to consider that the test could ever be a problem.

The story of an elderly Chinese man is found in a pre-publication medical article (https://www.researchsquare.com/article/rs-23197/v1):

> *A 68-year-old man was admitted due to fever, muscle pain, and fatigue. He was initially diagnosed with COVID-19 according to two consecutive positive results for SARS-CoV-2 RNA plus clinical symptoms and chest CT findings, and was discharged from hospital when meeting the discharge criteria, including two consecutive negative results. He was tested positive for SARS-CoV-2 RNA twice during the quarantine and was hospitalized again. He was asymptomatic then, but IgG and IgM [antibodies, with IgG indicating immunity] were both positive. He was discharged in the context of four consecutive negative test results for SARS-CoV-2 RNA after antiviral treatment. However, he was tested positive once again on the 3rd and 4th day after the second discharge, although still asymptomatic. IgG and IgM were still positive. After antiviral treatment, the results*

*of SARS-CoV-2 RNA were negative in three consecutive retests, and he was finally discharged and quarantined for further surveillance.*

The most disturbing thing about this article is that, at no point, did the authors raise the possibility of false positive test results. Perhaps the unnamed 68-year-old man would disagree, arguing that his life being turned upside down, being forced to take drugs while healthy, and being isolated from his family was more disturbing.

## More Information

For more information, discussion and references, see David Crowe's critique of the COVID-19 pandemic theory at: http://theinfectiousmyth.com/book/CoronavirusPanic.pdf

## Summary Table

### Key

- Cq/Ct. Test notes that sufficient RNA must be detected before this number of cycles in order to be considered positive.
- Positive Definition. (1/1) Looks for one genome segment. (1/2) Looks for two genome segment, but positive is declared if either one is found. (2/2) Both genome segments must be present. (2/3) Looks for three segments, and any two qualify as positive. (3/3) All three segments must be positive. Names such as N, E, S, ORF1ab, RdRp are segments of the coronavirus genome ('genes') that usually amount to 1% of the total, which is about 30,000 bases.
- Limitations. These are words taken directly from the test label.

| **Test name and URL** | **Cq/Ct** | **Positive definition** | **Limitations** |
|---|---|---|---|
| Abbott RealTime SARS-CoV-2 | 40 | (2/2) N1+, N2+ | 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. Positive results do not rule out bacterial infection or co-infection with other viruses…The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs [on the performance of this test] have not been evaluated… Due to the high sensitivity of the assays run on the instrument, contamination of the work area with previous positive samples may cause false positive results |
| Accula Test for SARS-CoV-2 | n/s | Not specified, just a coloured line with no meaning assigned. | Test results should be interpreted in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests performed… Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection… Cross-reactivity with respiratory tract organisms other than those listed in the Analytical Specificity Study may lead to erroneous results… Analyte targets (viral nucleic acid) may persist in vivo, independent of virus viability… contamination of the work area with previous samples may cause false positive results |
| Applied Biosystems TaqPath™ COVID-19 Combo Kit | 40 | (2/3) ORF1ab+, N+, S+ | False-positive results may arise from: (1) Cross contamination during specimen handling or preparation (2) Cross contamination between patient samples (3) Specimen mix-up (4) RNA contamination during product handling… The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated… Positive results are indicative of the presence of SARS-CoV-2 RNA |

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| ATILA iAMP COVID-19 | 30 | (1/2) ORF1ab+ OR N+ | The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs [on this test] have not been evaluated |
| AvellinoCoV2 test | 40 | (2/2) N1+, N3+ | None |
| BD BioxGX | n/s | (1/2) N1+ OR N2+ | Reliable results depend on proper sample collection, storage and handling procedures… |
| BGI Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV | 38 | (1/n) FAM+ (gene not specified) | False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology… Failure to take proper precautions when handling the positive control could result in a false positive result… False-positive results may arise from: (1) Cross contamination during specimen handling or preparation (2) Cross contamination between patient samples (3) Specimen mix-up (4) RNA contamination during product handling… The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated… A positive result indicates the detection of nucleic acid from the relevant virus… Nucleic acid may persist even after the virus is no longer viable |
| BioFire COVID-19 Test | n/a | (2/3) ORF1ab, ORF1ab, ORF8 (presumably two different regions of ORF1ab) | There is a risk of false positive and false negative results caused by improperly collected, transported, orhandled samples. |
| CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel<br>https://www.fda.gov/media/134922/download | 40 | (2/2) N1+, N2+ | "Positive and negative predictive values are highly dependent on prevalence…False positive test results are more likely when prevalence is moderate to low…Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms." |
| Roche Cobas SARS-CoV-2 | n/s | (1/1) Target1+ | Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection… False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing. |
| Curative-Korva SARS-Cov-2 Assay | 36 | (1/1) N+ | None |
| Fosun COVID-19 RT-PCR | 37 | (2/3) ORF1ab+, N+, E+ | The positive result detected by this kit can't indicate whether there is virus in vivo… The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses…the contamination of laboratory |

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| | | | environment and reagent, or cross contamination during specimen treatment may lead to false positive result |
| GeneFinder™ COVID-19 Plus RealAmp | 40 | (1/2) RdRp+ OR N+ | False positive and false negative results can be caused by poor specimen quality, improper specimen collection, improper transportation, improper laboratory processing, or a limitation of the testing technology… False-positive results may arise from: (1) Cross contamination during specimen handling or preparation; (2) Cross contamination between patient samples; (3) Specimen mix-up; (4) RNA contamination during product handling… The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated… A positive result indicates the detection of nucleic acid from SARS-CoV-2… Nucleic acid may persist even after the virus is no longer viable. |
| GenMark ePlex SARS-CoV-2 Test | n/s | Not specified | The performance of this test has not been established for immunocompromised individuals… The performance of this test has not been established for patients without signs and symptoms of respiratory infection… Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient… Viral nucleic acids may persist in vivo, independent of viability… There is a risk of false positive or false negative values resulting from improperly collected, transported, or handled samples… There is a risk of false positive results due to contamination of the sample with target organisms, their nucleic acids, or amplicons… There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract |
| GenoSensor COVID-19 RT-PCR | 40 | (3/3) ORF1ab+, E+, N+ | False positive results may be caused by: (1) Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template. (2) Unsuitable handling of amplified product. |
| Gnomogen COVID-19 RT-Digital PCR | n/s | Not sure what genes are used | False positive test results are more likely when prevalence is moderate to low… |
| InBios Smart Detect SARS-CoV-2 rRT-PCR | 39 | (2/3) N+, E+, ORF1b+ | False positive results may happen from cross- contamination between patient samples, specimen mix-up and RNA contamination during product handling…Detection of SARS-CoV-2 RNA indicates presence of viral RNA, however this does not confirm that SARS-CoV-2 is the causative agent of clinical symptoms |
| Ipsum Diagnostics COV-19 IDx assay | 35 | (1/1) N1+ | |
| Logix Smart™ Coronavirus Disease 2019 | 45 | (1/1) RdRp+ | Appropriate specimen collection, transport, storage, and processing procedures are required for optimal results…As with any diagnostic test, results of the Logix Smart COVID-19 kit are to be interpreted with consideration of all clinical and laboratory findings…report to [manufacturer and FDA] any suspected occurrence of false positive or false negative results. |

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| Lyra® SARS-CoV-2 Assay | 31<br>40 | (1/n) RNA detected (other machines)<br>(1/n) RNA detected (Roche) | Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, nor are the causative agents for clinical symptoms… The assay performance was not established in immunocompromised patients… There is a risk of false positive values resulting from cross-contamination by target organisms their nucleic acids or amplified product, or from non-specific signals in the assay |
| Luminex ARIES SARS-CoV-2 Assay | n/s | (1/2) ORF1ab+ OR N+ | Analyte targets (viral sequences) may persist in vivo, independent of virus viability… All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data and other data available to the clinician evaluating the patient… The detection of pathogen nucleic acids is dependent upon proper specimen collection, handling, transportation, storage and preparation… There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay… The performance of this assay was not established in immunocompromised patients… The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions… The performance of this device has not been evaluated for patients without signs and symptoms of infection… Cross-reactivity with respiratory tract organisms other than those tested can lead to erroneous results |
| Maccura SARS-CoV-2 Fluorescent PCR | 40 | (1/2) N+ OR E+ | the SARS-CoV-2 Fluorescent PCR Kit may cross-react with SARS-coronavirus… There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay… Analyte targets (viral sequences) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms… The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions… The performance of this device has not been evaluated for patients without signs and symptoms of infection [or] in immunocompromised patients… The performance for some viruses and subtypes may vary depending on the prevalence and population tested |
| NeuMoDx™ SARS-CoV-2 Assay | 40 | (1/2)<br>Nsp2-gene Ct 4..11, EPR ≥1.2, EP ≥ 700<br>OR<br>Nsp2-gene Ct 12..40, EP ≥ 700<br>OR<br>N Ct 4..11, EPR ≥1.5, EP ≥ 1000<br>OR<br>N Ct 12..40, EP > 1000 | Erroneous results could occur from improper specimen collection, handling, storage, technical error, or specimen tube mix-up…A positive result does not necessarily indicate the presence of infectious SARS-CoV-2. However, a positive result for both targets is indicative of the presence of SARS-CoV-2 RNA |

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| New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)- PCR Diagnostic Panel<br>https://www.fda.gov/media/135847/download | 40 | (2/2) N1+, N2+ | "Positive and negative predictive values are highly dependent on prevalence…False positive test results are more likely when prevalence is moderate to low…Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms." |
| Panther Fusion SARS-CoV-2 | n/s | n/s | A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable. |
| Perkin-Elmer New Coronavirus Nucleic Acid | 43 | (1/2) N+ OR ORF1ab+ | Inappropriate specimen preparation and operation may lead to inaccurate results… amplicon contamination can be avoided only by strictly following the instructions of PCR laboratories… The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated… |
| Primerdesign COVID-19 genesig RT-PCR assay | n/a | (1/n) FAM+ (gene unspecified) | False positive results may be caused by: (1) Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template. (2) Unsuitable handling of amplified product… All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms… Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions |
| QIAstat-Dx Respiratory SARS-CoV-2 Panel | n/a | (1/2) Rdrp+ OR E+ (one fluorescence channel) | The performance of this test has not been established for immunocompromised individuals… The agent detected may not be the definitive cause of the disease… Viral and bacterial nucleic acids may persist in vivo, even if the organism is not viable or infectious. Detection of a target marker does not imply that the corresponding organism is the causative agent of the infection or the clinical symptoms… Detection of viral and bacterial nucleic acids depends on proper sample collection, handling, transportation, storage and loading… The performance of this test has not been established in individuals who received influenza vaccine… False positive test results are more likely during periods when prevalence is moderate or low… |
| QuantiVirus™ SARS-CoV-2 | n/s | (2/3) ORF1ab+, N+, E+ | Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences… False Positive results may arise from the contamination during specimen handling or preparation, or between patient samples… |
| Quest Diagnostics SARS-CoV-2 RNA, Qualitative Real-Time RT-PCR | 40 | (2/2) N1+, N3+ | Positive and negative predictive values are highly dependent on prevalence…False positive test results are more likely when prevalence is moderate to low. |
| Sciencell SARS-CoV-2 Coronavirus Real-time RT-PCR | 40 | (1/2) N1+, N2+ | A false positive result may arise from cross contamination during specimen handling or preparation, or between patient samples… The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs |

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| | | | have not been evaluated… Results from the ScienCell™ SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit should be used as an adjunct to clinical observations and other information available to the physician… |
| Simplexa™ COVID-19 Direct | n/s | (1/2) ORF1ab+ OR S+ | As with other tests, false-positive results may occur. |
| Xpert Xpress SARS-CoV-2 | n/s | (1/1) N2+ (E+ presumptive positive) | Specimen stability under shipping conditions other than those recommended has not been evaluated… Positive results are indicative of presence of SARS-CoV-2-RNA |

## Summary of Common Limitations

| **Name** | **Description** |
|---|---|
| RNA not Virus | Positive test results indicate that RNA is present, not necessarily a virus. |
| Clinical Information | Clinical information is necessary to complete a diagnosis. |
| Other substances | The test has not been validated with commonly used drugs or vaccines, which may change the results. |
| Sample | Sample collection, transportation and storage may introduce errors. |
| PPV | False positives are more likely to occur when testing a population with few infected people. |
| Cause | Presence of viral RNA does not prove that it is the cause of any symptoms present |
| Contamination | Cross contamination may result in false positive results. |
| Coronavirus | The test may be positive due to the presence of other coronaviruses |
| Dead virus | RNA from the virus may persist after infection and produce false positive results. |
| False Positive | False positives may occur due to contamination or ‘non specific signals’ |
| Subgroups | This test has not been tested on certain types of people (e.g. the immune compromised, the asymptomatic). |
| Mixup | Specimen mixup can cause false positives (and false negatives) |