

**Coronavirus Diagnostics: An FAQ**  
Advanced Diagnostics & Therapeutics  
University of Notre Dame

**Coronavirus vs. COVID-19: are they the same?**

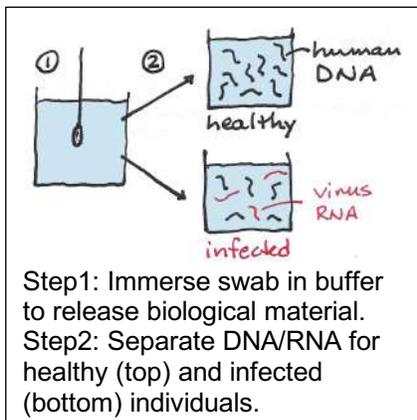
COVID-19 is the illness caused by coronavirus infection. Infection with coronavirus can lead to a variety of symptoms. It is possible to be infected with coronavirus without developing the symptoms associated with COVID-19. You may also develop COVID-19 with symptoms ranging from mild or severe.

**Coronavirus Testing**

*What question does the test address?* The coronavirus test (the “test”) is a DNA-based test to determine whether an individual has the coronavirus. Because this is a “threshold-based” test, you receive a yes/no answer.

**How the test works**

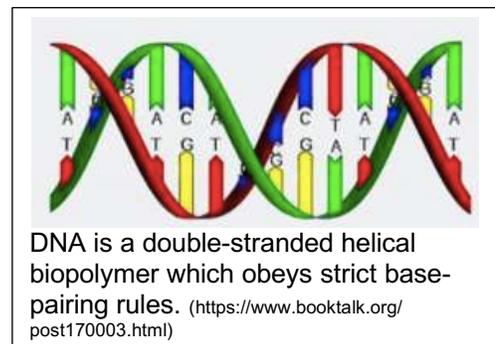
*What happens during the test?* The test begins with a throat or nose swab administered at the test site by trained personnel. The throat swab is similar to ones that test for strep throat or other viral infections. Once the throat or nose swab is completed, you will typically be asked to return home and self-isolate until the result of the test is known.



*What happens to the cotton swab?* The cotton swab is rinsed with a simple solution (called a buffer) to release the biological material that it contains. After separating any human cells in the material, the remaining fluid is treated with an enzyme (proteinase K) that releases any RNA contained within a virus particle. This works because the virus has a protein shell that contains the RNA. At this point the fluid contains cell-free DNA and RNA from you (*i.e.* human DNA) if you are not infected with coronavirus (See figure, left “healthy”). If you are infected with coronavirus, it contains human DNA and RNA and viral RNA (see “infected”). The RNA is isolated from the fluid solution and is converted into DNA using a reverse transcriptase enzyme before proceeding.

*Why DNA?* DNA is a remarkable molecule, and it is the basis of the coronavirus test. DNA consists of two twisted, or braided, strands

which form a helix. Each strand is a linear polymer in which each repeat unit is labeled with one of four nucleobases: A, C, T, or G. The other strand consists of an exactly complementary set of bases, but just like the dances we all remember from junior high, only certain pairings are allowed: A's always combine with T's, and C's always combine with G's. RNA is closely related to DNA, although it is single stranded and uses the nucleobase U (uracil) instead of T. Because DNA is more stable than RNA, it can be more readily manipulated, making DNA a better choice for the detection strategies below.



*What is the basic strategy of the test?* In principle, we would like the test to detect the presence of any coronavirus DNA at all (down to a single copy of coronavirus DNA). However, our tests are not sensitive enough to detect a single strand of DNA. To circumvent this problem, the coronavirus test takes advantage of three important molecular strategies.

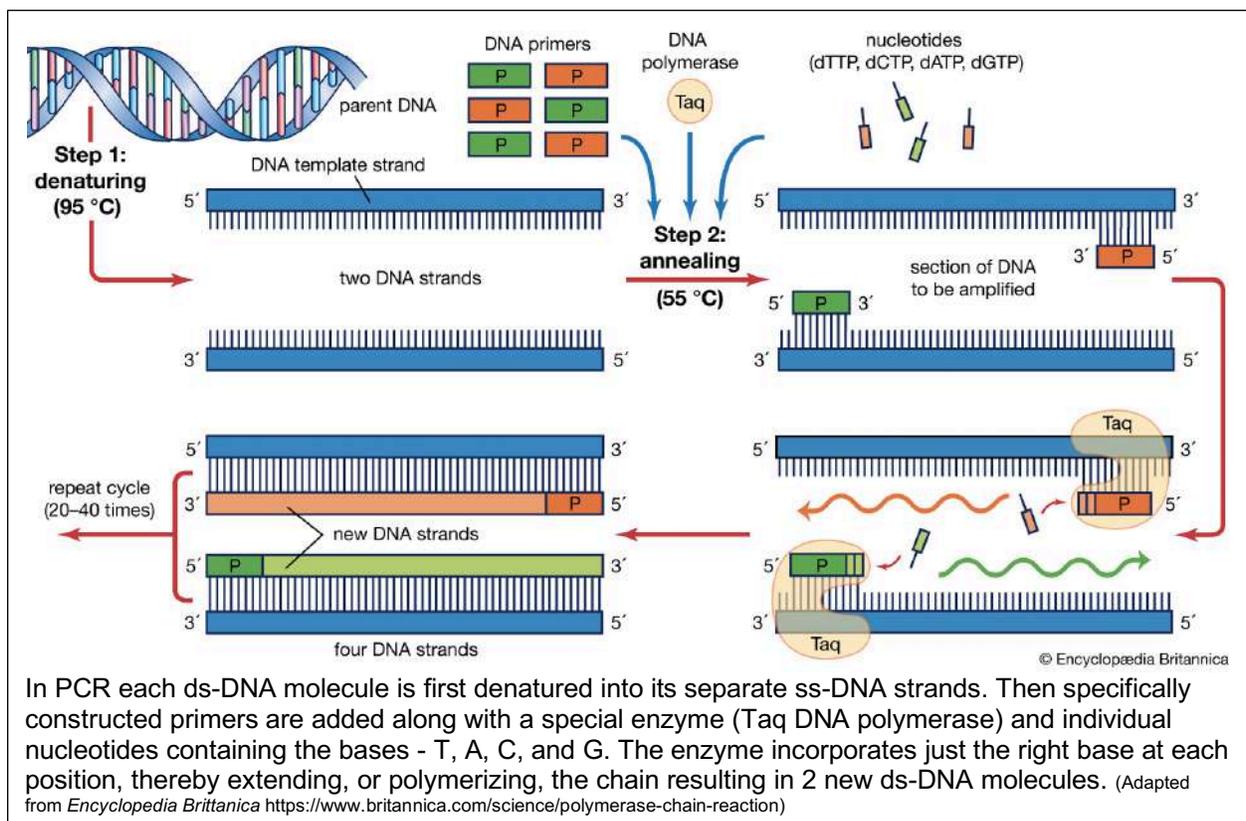
- **Amplification:** DNA can be *amplified* in a scheme called polymerase chain reaction, or PCR.

Just 20 cycles of PCR produce more than a million-fold amplification! (See below).

- **Specificity:** The second technique is based on the ability of specific DNA strands to recognize and bind to their complements. For example, a piece of DNA containing the 4 bases A-C-T-G in sequence (an oligonucleotide) would bind to any DNA that has T-G-A-C (See the DNA figure above).

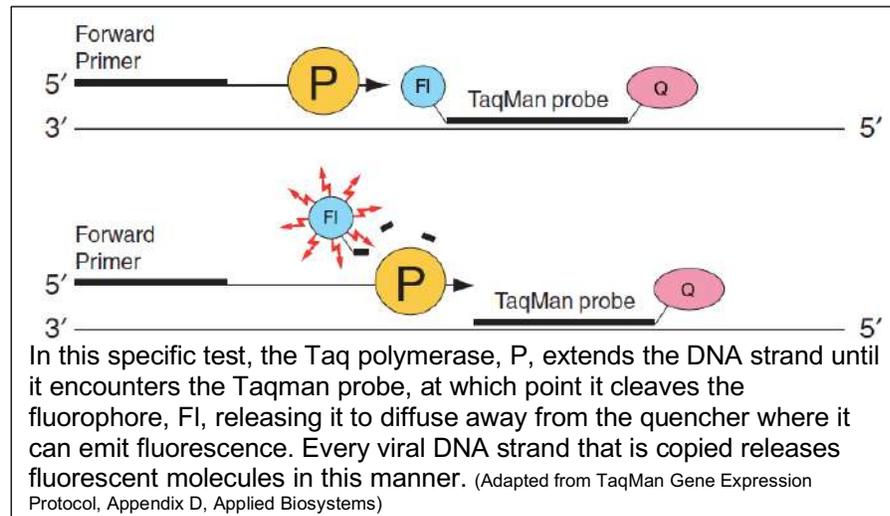
**Sensitivity:** The third technique relies on generating measurable fluorescence signal by releasing one molecular unit of fluorescence with each new DNA strand, thus amplifying the fluorescence along with the DNA until it reaches a level where it may be *measured reliably*.

*Amplification: How is DNA “amplified”?* In the 1990’s a transformative new technology, PCR, was developed, which made it possible to amplify DNA through a molecular copying scheme. The basic idea of PCR, which consists of a set of molecular processing steps in each “cycle” is that it turns a single copy of the DNA molecule into 2 copies. Then the cycle can be repeated turning 2 into 4, and 4 into 8, etc. The scheme for amplification is shown in the figure below.



*Specificity: How is viral DNA distinguished from the human DNA which is also in the sample?* This works because it is relatively straightforward to synthesize DNA strands that contain specific sequences of 20-30 bases. The short pieces of DNA/RNA used for this purpose are called ‘probes’, and they are *highly specific* for recognizing their complements. For example, the probability of encountering a perfect match to a chosen 2-base sequence, say A-G, by chance is 1 in 16 ( $4 \times 4 = 16$ ). Using similar logic, the probability of matching a 23-base sequence by chance is 1 in 70 trillion. The coronavirus test is based on the fact that the coronavirus RNA was sequenced very quickly after appearance of the disease, so the complete genetic sequence of the coronavirus RNA is known. Thus, researchers could identify specific sequences of ~20 bases that were contained in the viral genome but not in humans. DNA probes were constructed that were complementary to these viral sequences. For example, one of the CDC probes is ACC CCG CAT TAC GTT TGG ACC. This probe binds only to viral DNA.

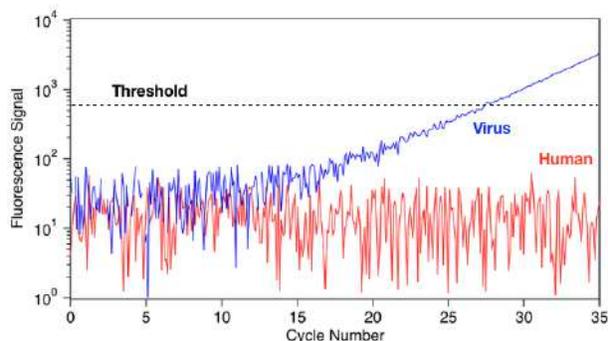
**Sensitivity: How is the fluorescence generated?** The actual measurement of amplified DNA uses a molecular beacon scheme - so named, because like a beacon the sample is dark until the beacon turns on, but when it is turned on, it shines brightly.



Molecular beacons are constructed by placing a fluorescent molecule (FI in the figure) at one end and a quencher (Q) at the other end of a specific sequence of DNA bases that are complementary to the coronavirus target sequence. Because FI and Q are in close proximity, the quencher effectively extinguishes the fluorescence. However, when the DNA-extending polymerase reaches the

probe, it cleaves off the fluorescent molecule which can then diffuse away so that it is no longer extinguished.

Furthermore, because every viral DNA probe strand initially has a molecular beacon probe bound to it, every new strand that is synthesized by the PCR reaction releases a fluorescent molecule, and the fluorescence is amplified in concert with the DNA. Finally, the fluorescence signal is measured on a specialized instrument, and the signal is plotted in real time (thus the name real time PCR). If the sample contains coronavirus DNA, then the fluorescence exceeds a predetermined threshold value at some point well before the cycling limit (typically 35 cycles) is reached.



Typical fluorescence curves generated from virus-containing (blue) and human-only (red) samples.

### **How the results are interpreted**

Although the test is highly specific and accurate, it is not perfect. While researchers strive to produce the best test possible, there are two kinds of errors that can occur.

### **What is a false positive and what are its implications?**

A false positive is a test that is “positive” for an individual who does not have the virus.

### **What is a false negative and what are its implications?**

The much more serious error is a false negative, which is a test that gives a “negative” result for an individual who has the virus. In this case, an individual might believe they are in the clear, when in fact they have the coronavirus. This is why the standard for clearance of the disease in a previously infected individual is multiple (at least 2) consecutive negative tests.

### **Additional Reading**

Wired Magazine: <https://www.wired.com/story/everything-you-need-to-know-about-coronavirus-testing/>

CDC: <https://www.cdc.gov/coronavirus/2019-ncov/index.html>