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Additional material

How to understand a COVID-19 test result

Scientific background

How do the COVID-19 tests work?

At the beginning of the pandemic, blood samples were commonly tested for antibodies produced by the patient themselves against the virus. The disadvantage of this test type is that a person who has recently been infected may not yet have antibodies, and on the other hand, antibodies can persist in the blood long after the infection is over. However, these tests could be a useful means of establishing who has immunity following infection or vaccination.

The PCR tests discussed in the article work by detecting the presence of the SARS-CoV-2 virus, which causes the disease COVID-19, in a patient sample, usually taken from a nose and throat swab. Specifically, these tests detect short regions of the viral RNA genome using a technique called reverse-transcriptase polymerase chain reaction (RT-PCR). This requires sophisticated equipment so samples must be sent to a laboratory for analysis, but these tests can be very sensitive and detect the virus at low levels.

A third major kind of COVID-19 test is the more recently developed laminar flow tests, which are a kind of immunoassay. These test for the presence of viral proteins by using manufactured antibodies that bind specifically to a particular viral protein (antigen). They work in a similar way to pregnancy tests and give an immediate result without requiring complex equipment. However, they may not detect the virus when it is present at low levels.

Finally, a number of variations on these approaches have been developed and tested, for example using isothermal amplification instead of PCR, or different detection methods.

These different tests types have different advantages and disadvantages, and each developed test will have different accuracy values, so different tests may be most appropriate in different contexts.

What is RT-PCR?

RT-PCR is based on the polymerase chain reaction (PCR), which enables the amplification of a piece of DNA with a specific sequence by using an enzyme called DNA polymerase and primers, which are short pieces of DNA complementary to the sequence you are testing for, to make huge numbers of copies. Only DNA sequences that match the primers will be amplified, and in each cycle, all target DNA fragments present are copied once, so their number doubles with each cycle (exponential growth). This means that millions of copies can be produced within a few hours, thus the technique can detect even very low starting levels of DNA.

So what about the RT part? SARS-CoV-2 is an RNA virus, which means that it has an RNA rather than a DNA genome. PCR only works with DNA, so to detect RNA, it must first be converted into DNA through reverse transcription. According to the 'central dogma' of molecular biology, DNA is transcribed into RNA (which is translated into protein). However, some organisms are able to do the reverse; they have enzymes than can reverse transcribe RNA into DNA. RT-PCR allows the amplification of RNA by using one of these reverse transcriptase (RT) enzymes to first make DNA copies of the RNA, before proceeding with the PCR.

Finally, you might have read reports where 'viral loads' or the levels of the virus in a sample or patient are reported. This is possible because most of these RT-PCR tests actually use quantitative PCR (qPCR) rather than standard PCR. qPCR is a variant of PCR where the progress of the amplification can be monitored over time through the use of fluorescent dyes or probes. This allows the starting DNA amount, which should be proportional to the number of viral particles in the sample, to be calculated.

A nice video explanation of the COVID-19 RT-PCR test can be found here:
https://www.youtube.com/watch?v=Vd38iS_W7ww&ab_channel=DNALearningCenter

Discussion points

- If you were running a testing program, what factors would you need to consider (e.g., accuracy, speed, expense)?
- What are the advantages and disadvantages of the different test types discussed?
- Are there some situations where a less accurate but cheaper/faster test might be the better choice? Does it make a difference whether inaccuracy is due to false positives (low specificity) or false negatives (low sensitivity)?

- What other medical tests could the discussed concepts of sensitivity, selectivity, and pre-test probability? Answer – essentially any test with a yes/no result. Pregnancy tests are an obvious one.
- For a highly infectious disease like COVID-19, false negatives are a very serious problem since it could lead to infected people thinking they are negative and failing to isolate and infecting others. Are there diseases where false positives might be just as bad or worse? Clue: What about non-infectious diseases with treatments that have serious side effects, like chemotherapy or surgery.
- What virus proteins might a laminar flow test be able to detect? This can be linked to basic virus biology. Students may know that the viral particle is assembled from capsid proteins, for example.