TESTING FOR COVID-19

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How do we detect COVID-19 infection? What kind of samples are needed to test infection? What is the likelihood of a test result being inaccurate? How do molecular and antigen tests work? When are antibody tests effective? Which tests would be most effective for contact tracing, and which for population-wide screening?

COVID-19 is caused by the virus SARS-CoV-2, which belongs to a family of coronaviruses that infect the respiratory tract (see **Fig. 1**). It can be diagnosed by testing for the presence of genetic material (molecular tests) or proteins (antigen tests) specific to the virus, or the body's response (antibody test) to infection (see **Fig. 2**).

For any test to be useful, it must be **specific**, detecting only the element (molecule, antigen or antibody) of interest. It should also be **sensitive**, giving positive results even when the relevant element is present at low concentrations in the sample. In theory, a perfect test would have a **specificity** (or true negative rate) and **sensitivity** (or true positive rate) of 100% (see **Table I**). In practice, there are no perfect tests. A test with a sensitivity of 99% will miss the presence of the antigen or antibody of interest in 1 out of 100 samples tested (1 **false negative**). Similarly, a test with a specificity of 95% will incorrectly identify the presence of the antigen or antibody in 5 of every 100 samples (5 **false positives**).

Table I. What are false negatives and false positives?

	Has COVID-19	COVID-19 free
Tests positive for COVID-19	True positive	False positive
Tests negative for COVID-19	False negative	True negative

Molecular tests

Designed to detect the presence of the genetic material of SARS-CoV-2 in a swab sample, these tests are based on a method called **Reverse Transcription Polymerase Chain Reaction** or RT-PCR (see **Box 1**). With a sensitivity and specificity that is close to 100%, molecular tests set the gold standard in COVID-19 testing.

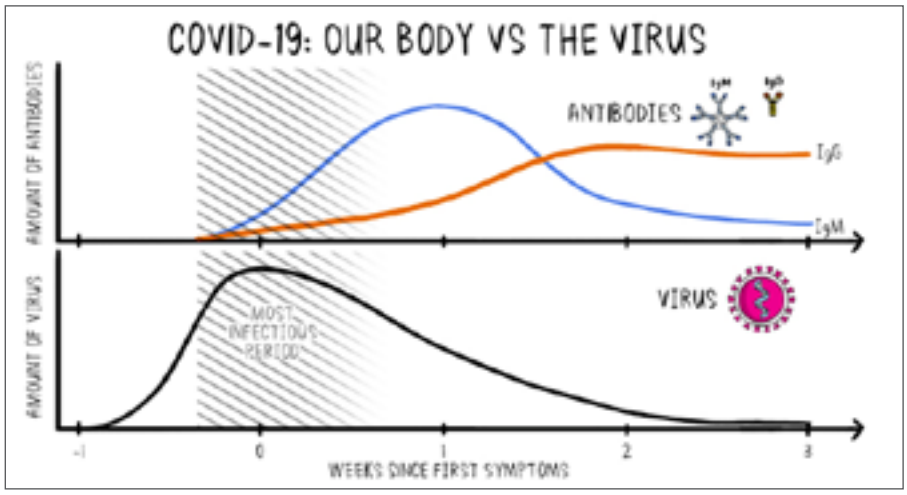

Fig. 1. A simplified view of COVID-19 infection. On entry into a host cell, SARS-CoV-2 replicates and multiplies. The number of viruses in the body increase with time, and peaks at around the time an infected person starts showing symptoms of disease. Our immune system responds to the virus by making two kinds of antibodies — called IgM (Immunoglobulin M) antibodies & IgG (Immunoglobulin G) antibodies. Both begin to appear at about the same time as the first symptoms of disease. IgM antibodies, which lock on to the virus particles and prevent them from entering cells, peak a week later. IgG antibodies, which retain 'memory' of the virus and protect us against reinfection, peak about two weeks after symptoms appear.

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Molecular tests are of two kinds:

(a) Real-time RT-PCR: This is the most common molecular test. 'Real-time' refers to the fact that the concentration of a certain molecule (like virus-specific DNA) is detected (using fluorescent probes) as the test (like the RT-PCR) is being run. To do this, some of the building blocks added to build new strands in the DNA amplification step are labelled with fluorescent tags to act as probes. As the probes become part of new copies of the virus-specific DNA, their tags are released into the solution. The fluorescence of the solution is measured at the end of each cycle. When it reaches a level that is known to correspond to 35 billion copies of DNA, the test is considered positive for infection. The number of cycles (Ct)

Fig. 2. Different samples are collected for different tests.

(a) A nose or throat swab of potentially infected cells is used as a sample for molecular or antigen tests.

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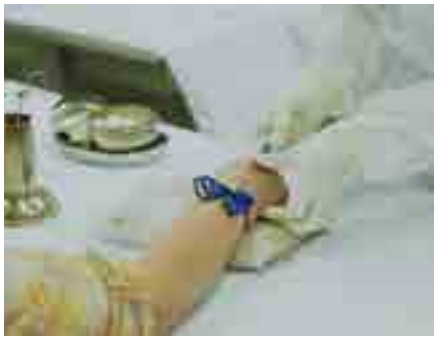

(b) A blood sample is collected for antibody tests.

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Box 1. How do molecular tests work?

In the first step of a molecular test, all the RNA in a swab sample (cellular and virus) is reverse transcribed into complementary DNA molecules. In the second step, only DNA sequences specific to the virus are amplified through a polymerase chain reaction (see **Fig. 3**). The specificity of this step is ensured by the use of specific primers. **Primers** are short stretches of DNA that initiate replication by binding to a complementary sequence in the template. Molecular tests for SARS-CoV-2 use primers for sequences from two genes — the gene for the E protein, and the RdRp gene that makes **R**NA **d**ependant **R**NA **p**olymerase enzyme. Sequencing of the SARS-CoV-2 genome shows that both genes are unique to the virus and slow to mutate. Primers for gene sequences from related virus families are used as controls.

If the virus is present in the swab, these primers will be able to initiate the replication of both gene sequences. Only samples that test positive for both genes are considered to be true positives for COVID-19. Samples that test negative for the gene for E protein are considered to be true negatives for the infection. Each of the 35-40 PCR cycles will double the number of these sequences, amplifying their concentration manyfold. This allows easy detection of even trace amounts of the virus in a swab sample.

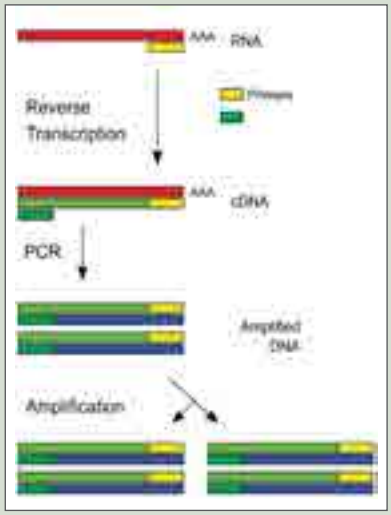

Fig. 3. The RT-PCR method.

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required to reach this number tells us what the original viral load was. In India, Ct < 35 is positive, Ct > 35 is negative, and Ct = 35 suggests that the test needs to be repeated. This test is highly specific, giving no false positives. It is also very sensitive, testing positive even if a single virus particle is present in the swab sample. But it is time consuming (results take a day) and expensive. The sample has to be collected and handled carefully by specially trained health professionals to minimise risk of contamination. It has to be transported to a laboratory which has uninterrupted power supply and air-conditioning for maintenance of the real-time RT-PCR machine. Sample preparation takes about 2-4 hours, and the PCR cycles take about 4-8 hours to run. Most accredited laboratories can run about 200-300 tests per day, working around the clock. Delays in test results impact the trace (contacts of an infected person), test (each contact), quarantine (those who test positive) protocol that many countries have adopted to reduce spread of infection. Contacts may need to be quarantined before their test results can be obtained to confirm the necessity of this measure.

(b) TrueNat tests: This test uses chip-based, indigenously developed, battery operated, portable RT-PCR machines that were originally designed for contact testing for TB and HIV. The swab is treated with a lysis buffer (lysis = break up) to inactivate the virus and added to micro-PCR chips that are preloaded with the necessary reagents. Each machine can have 1, 2 or 4 channels, and each channel can be used to test an independent sample. Since these tests can be run where the samples are collected, the results are available sooner. This allows higher testing rates, and more localised responses to the spread of infection.

Rapid Antigen Test (RAT)

Designed to detect the presence of virus proteins **(antigens)** that are capable of producing an immune response in our body, this test is based on a method called **Lateral Flow Immunoassay** or LFIA (see **Box 2**).

This test is relatively inexpensive and quick (results are available in about 30 minutes), making it particularly suitable for contact tracing. On the other hand, antibodies against the S protein of SARS-CoV-2 can bind to proteins of

Box 2. How does RAT work?

An LFIA is designed to be used in a dipstick format or in a housed cassette (see **Fig. 4**). Both involve a test strip that has a sample pad, a conjugate pad, and a nitrocellulose membrane. In a RAT test, the conjugate pad has a mixture of two sets of antibodies (called primary antibodies) — one set can bind to the antigen of interest (the S protein of the SARS-CoV-2 virus) while the other can bind to a control protein that is universally found in human blood samples. Both sets of primary antibodies are conjugated to dyes. The nitrocellulose membrane has two bands of immobilised (or capture) antibodies. One of them, called the test band, has capture antibodies against a different part of the antigen of interest. Similarly, the other band, called a control band, has capture antibodies against a different part of the control protein.

The antigens in a swab or sputum (coughed up) sample flow laterally across the strip through capillary action. When they reach the conjugate pad, one set of primary antibodies bind to the control protein. If the swab contains infected cells, the other set of primary antibodies bind to the S protein. When the capture antibodies in the test band grab onto the S protein, the accumulation of dye molecules in this part of the strip causes the test band to colour up. Similarly, when the capture antibodies in the control band bind to the control protein, the control band colours up. A sample is considered positive for the virus when colour appears in both the test and control bands. It is considered negative for the virus when colour appears only in the control band. If no colour is produced in either band, then the test is considered invalid.

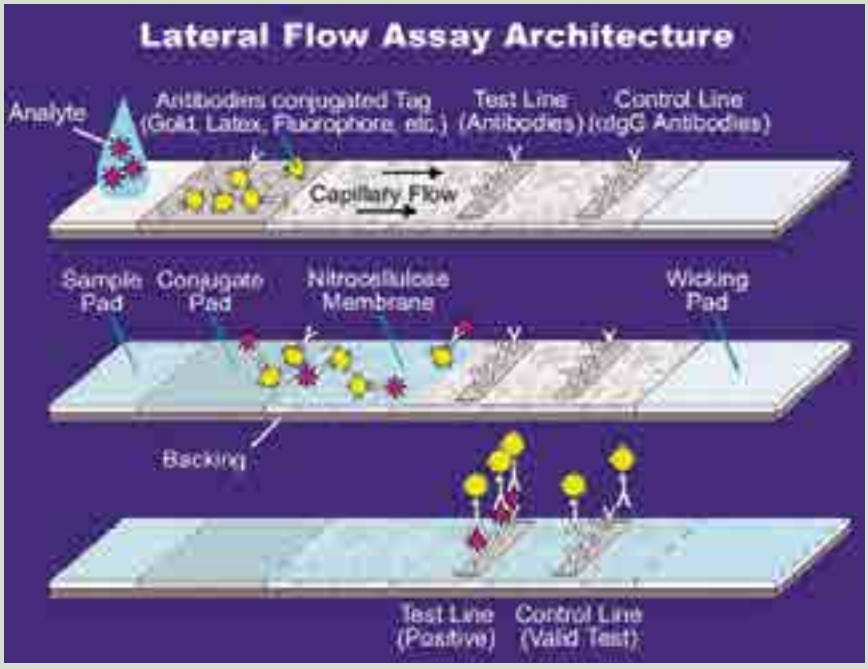

Fig. 4. The design of a lateral flow immunoassay.

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other coronaviruses, increasing the likelihood of false negatives. Also, since it does not involve any kind of amplification, the antigen needs to be present in sufficient quantities in a swab for it to be detected. As a result, the specificity of this test can vary from 80-90%, and sensitivity can range from 34-90%. This means that a molecular test is needed to confirm a negative RAT result. But a positive RAT result is considered a true positive for the virus. Such patients (about 50% of those found infected according to some reports) can get into isolation faster, reducing the chances of spread of the disease.

Antibody Tests

Doctors and scientists studying a pandemic explore several questions — how many people are infected by the virus at any given point of time? How many remain asymptomatic? How many develop symptoms early, and how many have delayed onset of symptoms? Can someone who has recovered from a COVID-19 infection catch the infection again? In addition, scientists use contact tracing to find out how the virus spreads. This knowledge allows health care teams to advise governments on controlling spread of the disease, and advise people on measures to remain safe. Molecular and antigen tests can only indicate current infections. Antigen tests generally pick up signs of infection in only those who have severe symptoms. But we know that many of those who are infected may be asymptomatic or have such mild symptoms that they never get tested. Antibody tests help us develop a better understanding of these aspects of the pandemic.

Antibody tests are designed to detect the presence of the IgM and IgG antibodies in blood serum or plasma samples. They are of two kinds:

- The most common antibody test is based on the **Enzyme Linked Immunosorbent Assay** or ELISA technique (see **Box 3**). This test can detect the presence of virus-specific antibodies with a high degree of accuracy. But because it requires a lab setting, it is relatively time-intensive and expensive.
- A **rapid serology antibody test**, like a RAT, is based on the lateral flow immunoassay technique. It is quick (test results are available in 15 minutes), relatively inexpensive, and can be conducted at point-of-care. Since it can be used to screen a large number of samples at the same time, it is particularly useful in detecting the spread of the disease in a large population within a short span of time. Hence, the World Health Organisation (WHO) is facilitating this test to determine the exposure and spread of the disease worldwide.

Box 3. How does an ELISA work?

In an ELISA, part of the antigen of interest is synthesised and adsorbed onto a base, like a plastic strip or a plate with wells (see **Fig. 5**). In the most common form of the test, the blood sample to be tested is diluted with a buffer and added to the plate. The plate is incubated to allow time for virus-specific antibodies to attach to the antigen in the plate. It is then washed to remove all unbound antibodies.

A second set of antibodies, called capture antibodies, is added to the strip or plate. Each capture antibody is linked to an enzyme molecule. Since this second set of antibodies is capable of binding to virus-specific antibodies, it will link to any antibody-antigen complex on the plate. After washing to remove any unbound material, the substrate for the enzyme is added to the plate. The presence of the enzyme linked to the second set of antibodies will result in the formation of a coloured product. The intensity of colour produced is a measure of the antibody concentration in the blood sample.

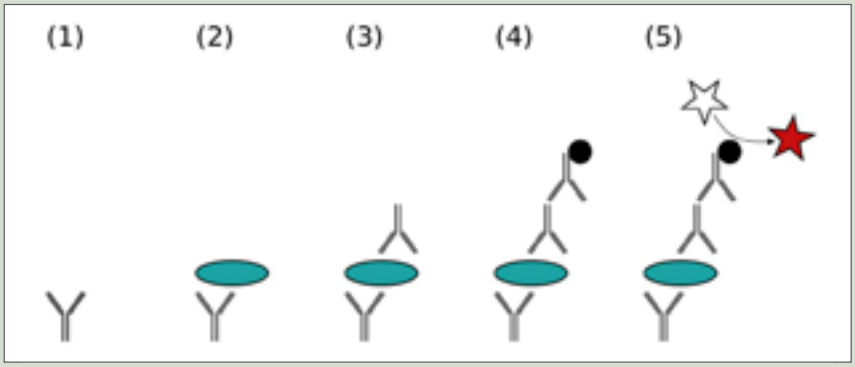

Fig. 5. The design of a 'sandwich' ELISA.

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Parting thoughts

Molecular and antigen tests can help control the pandemic by detecting the virus in early stages of infection, even before its symptoms become evident. Antibody tests help determine what proportion of a population has been exposed to the virus, and how long their protection against reinfection lasts. The more accurate these tests, the more effective they will be in combating the pandemic. Many testing kits are available, and newer ones are constantly being developed. But physical distancing, the use of masks, and frequent hand washing remain the most effective ways to minimise the spread of infection.

Key takeaways

- COVID-19 is diagnosed by testing for the presence of the virus or the body's response to infection.
- The usefulness of a test is measured by its specificity and sensitivity. No test is perfect.
- Molecular tests (real-time and TrueNat) detect genes specific to SARS-CoV-2 in nose or throat swabs using a method called Reverse Transcription Polymerase Chain Reaction (RT-PCR). These are the most accurate tests available.
- The Rapid Antigen Test (RAT) detects SARS-CoV-2 specific antigens in nose or throat swabs using a method called Lateral Flow Immunoassay. It allows quick screening of contacts of an infected person in the trace-test-quarantine protocol.
- Antibody tests detect IgM and IgG antibodies in blood serum or plasma samples using an Enzyme Linked Immunosorbent Assay (ELISA) or a Lateral Flow Immunoassay. These tests are particularly useful in population-wide screening, and identification of past infections.

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