SARS-CoV-2 and COVID Testing Technologies

Coronavirus disease (COVID-19) caused by SARS-CoV-2 has rapidly evolved into a global pandemic. Testing for COVID-19 is a foundational component of containment and mitigation strategies, as it allows the appropriate clinical management and public health interventions. This document describes the different types of tests and what they do.

There are 2 main modalities of current testing: **molecular-based tests** and **serology-based tests** (other types are being investigated but are in early stages of development).

Molecular-based tests (also called nucleic acid amplification tests), such as polymerase chain reaction (**PCR)** or Real-Time PCR (**RT-PCR**), detect the presence of viral genes and therefore detect active infection.

Serological tests measure the level of antibodies present in blood / serum / plasma and assess the overall immune response to the virus.

Both molecular-based and serology-based tests can be further divided according to the testing setting: **lab/hospital tests** and **Point of Care (POC) tests**. Hospital or lab tests require trained personnel as well as the analyzer platform (e.g., PCR machines, ELISA analyzers). POC tests are intended to be used by the end user, do not require significant training, and are very easy to use (some look like pregnancy tests).

	PCR		**Serological**	
Setting	Hospital/Lab	Point of Care	Hospital/Lab (ELISA test)	Point of Care
What does the test detect	Virus; can detect multiple viral genes which increases specificity		Patient Antibodies	Patient Antibodies or viral antigens (viral proteins)
Accuracy	High (Gold standard)	High	High	Low (to date)
Approved products (Cdn)	Yes	Yes	None	None
Patient samples	Nasal and throat swabs		Blood, serum, plasma	
Utility	Diagnosis: Indicates who *is* infected Can diagnose infection		Immune Status: Indicates who *has been* infected Can confirm infection The presence of antibodies *usually* correlates with immunity	
Throughput	High, particularly for multiplex or automated devices	Low	High	Low
Pros	High accuracy and througput	Rapid Can be used in remote/rural settings or non-healthcare settings like at the border	Immunoassays can provide historic information about viral exposure	Rapid Can be used in remote/rural settings or non-healthcare settings like at the border
Cons	Labour intensive Limited by number of capable laboratories Potential for false positives	Newer technology and less clinical data to support effectiveness	Not enough is understood about the role of antibodies in COVID-19 infection/immunity (the antibody response takes time to characterize); Only useful a few weeks after infection; Doesn't detect asymptomatic infection	Results not definitive; High cross-reactivity with other pathogens; not specific for SARS-CoV-2; High false negative; Risk of individuals self-diagnosing

Lab-Based Molecular Tests (PCR)

Nucleic acid amplification tests (NAT) such as polymerase chain reaction (PCR) or real-time polymerase chain reaction (**RT-PCR**) are the most sensitive test for detecting respiratory pathogens, including SARS-CoV-2.

What is the technology?

These tests detect the presence of viral genes and therefore detect active infection; results can be qualitative or quantitative. The specimens used for these assays are taken from the nasal and throat cavities as nasopharyngeal and oropharyngeal samples.

PCR uses "primers" designed to be specific to the genetic material to be detected. These primers bind to the complementary target DNA (SARS-CoV-2 in this case), just like a key and lock, and copies are made via exponential amplification in a series or cycles of temperature changes. With the rapid availability of genome sequences in early January 2020, laboratory-developed PCR tests for the detection of the SARS-CoV-2 were quickly developed. During the course of the outbreak, the PCR testing has been refined to an 80-85% specificity – i.e. the chance the test is detecting the virus.

What type of personnel is required?

Laboratory-trained personnel are required to run the assays. Training is also needed to collect the samples without contaminating the samples or others.

What is the throughput?

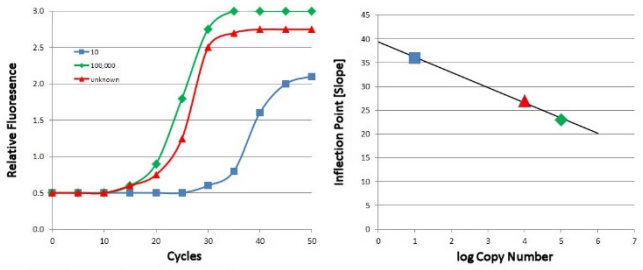
PCR can be done in standard formats (384 and 96 microwell plate) and typically take 4–6 hours to complete, but the logistical requirement to ship clinical samples means the turnaround time is 24 hours at best.

What information does it provide?

PCR assays detect the presence of the virus (SARS-CoV-2) and are therefore considered useful for diagnosis of infection.

How are results displayed?

The results are generated on a computer in software that is specific to the platform (machine) used to do the assay. The output for real-time PCR is Cycle Threshold (Ct) or number of cycles displayed on a graph: a low Ct indicates a high amount of virus in the sample as fewer cycles are needed to obtain high amplification; a high Ct indicates less virus. The Ct for the gene of interest (SARS-CoV-2 gene) is compared with the Ct of a housekeeping gene. The number of cycles can be converted into the amount of virus present in the sample when compared to a standard curve.



	Housekeeping gene			Gene of interest			
Sample	Ct1	Ct2	Average Ct	Ct1	Ct2	Average Ct	ΔCt
Control 1	17.19	17.16	17.18	30.57	30.53	30.55	13.38
Control 2	16.96	16.95	16.96	30.73	30.37	30.55	13.60
Control 3	17.07	17.15	17.11	30.76	30.82	30.79	13.68
Treated 1	18.04	17.95	18.00	26.11	25.54	25.83	7.83
Treated 2	17.99	17.91	17.95	25.70	25.56	25.63	7.68
Treated 3	17.90	17.86	17.88	25.64	25.74	25.69	7.81
Control average							13.55

Point of Care (POC) PCR

POC tests are needed to accelerate clinical decision-making and to take some of the workload off centralized test laboratories.

What is the technology?

Same as standard PCR

What type of personnel is required?

Anyone can be trained to run the assays. Training is needed to collect the samples without contaminating the samples or others.

What is the throughput?

The Spartan Cube can process one sample per hour. They anticipate a new release in a few weeks that will take only 30 minutes. The Abbott ID Now product can process one positive sample in 5 minutes.

What information does it provide?

Same as lab-based PCR

How are results displayed?

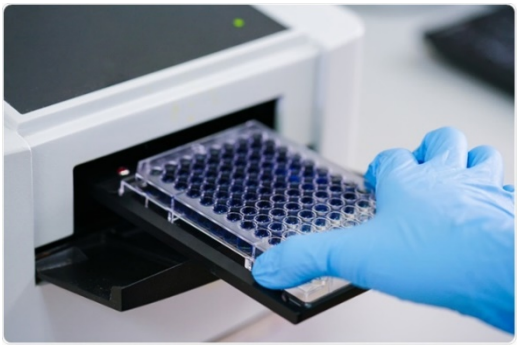
Same as lab-based PCR, in some form of computer readout that is specific to the platform (technology).

Lab-based Serology (ELISA)

Unlike molecular-based tests, which detect active infections, serological tests assess the overall immune response to the virus. As such, the results are more complicated than results from molecular-based tests (See Annex).

What is the technology?

Enzyme Linked Immunosorbent Assays (ELISA) are one of the most frequently used platforms for serological diagnosis. ELISAs use a color-based reaction with the antibodies in the patient's sample and a reader detects the intensity of the color to determine the presence or absence of antibodies.



What type of personnel is required?

Laboratory-trained personnel. Training is needed to collect the blood samples without contaminating the samples or others.

What is the throughput?

Many tests are performed in a 96-well ELISA microtiter plate and can run up to 92 patient samples in singlet, or 46 in duplicate depending on the preference of the laboratory. Tests can be performed manually or on automated instruments with additional validation.

They are considered high throughput assays because most utilise a 96-well plate format; many are automated and as are result. Depending on what the test is being used for, results may be obtained as quickly as about 24 hours if the test is done locally. However, there are some tests that may take days to weeks.

What information does it provide?

ELISAs are designed for the qualitative measurement of antibodies specific to a target virus in serum / blood / plasma. A positive result does not indicate the presence of the virus, nor of an active infection.

How are results displayed?

The results are generated on a computer in software that is specific to the platform (machine) used to do the assay. The absorbance of each sample is captured in a reader and the result is compared to the negative and positive controls.

Lateral Flow POC Serology

Point-of-care (POC) serology tests for SARS-COV-2 are generally qualitative immunochromatographic (lateral flow) assays that detect IgM and/or IgG antibodies from a fingerprick and can provide results in under 30 minutes. They are also often called rapid tests.

What is the technology?

These are essentially the same as home pregnancy kits. The lateral flow assay format — essentially a dipstick encased in a cassette — contains the capture reagents (either an antibody directed at a viral antigen or a viral antigen that is recognized by patients' antibodies) immobilized at defined locations on a nitrocellulose membrane, as well as labelled detector antibody that recognize the same target. A positive result, which is triggered by binding between the analyte, capture antibody, and detector antibody, is visible as a colored line. Two drops of blood from a pinprick is enough.

What type of personnel is required?

Anyone. However, they require adequate training to administer the test and interpret the result. Further, they need to be trained in managing the risk of infection with SARS-COV-2 and other bloodborne infections.

What is the throughput?

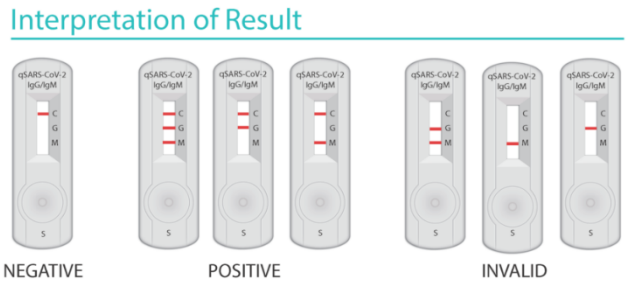
Extremely high because no expertise is needed and it only requires 15 to 30 minutes per sample (fingerprick)

What information does it provide?

These tests qualitatively assess the presence of IgG and IgM antibodies specific for SARS-CoV-2 from a patient sample or specimen. A positive result does not indicate the presence of the virus, nor of an active infection.

A description of the output – how are results displayed?

Results are displayed as a coloured line on a small cassette. These are not connected to a computer or other reader generally, though some products may be developed to be used in conjunction with a reader.



Annex – Serological Testing: Considerations

Rapid serological tests are very appealing for a number of reasons: they are easy to use, do not require specialised equipment or expertise and have very rapid turnaround times. The ease of use and quick turnaround time with POC assays make it an ideal testing modality in remote areas with limited access to centralized laboratory-based testing and/or limited local laboratory infrastructure, and in situations that would benefit from immediate triaging.

However, there are important considerations relating to: what they measure; the technology; and their application.

Considerations relating to what a serological test measures

Since serology tests do not detect virus, a positive or negative result does not determine whether a person is infectious. Positive results may be due to past or present infection with SARS-COV-2. It can take up to 7-12 days after symptom onset for antibodies to develop, therefore the use of serology tests in the early phase of infection is limited and can result in false negative results at a time when patients are most infectious (ie. a negative result does not rule-out infection). False negative results can also occur in elderly and immunocompromised patients. False positives may occur if the tests also cross react with antibodies from recent or past exposure to other coronaviruses, including human seasonal coronaviruses (HKU1, NL63, OC43, 229E) and SARS-COV-1. Any kits used need to be thoroughly evaluated for such cross reactivity before being used clinically.

Considerations relating to the technology of rapid serological tests

The performance of these tests are often inferior relative to laboratory-based assays, and therefore, significantly increase the risk of a false negative result. In addition, the performance of these assays has rarely been evaluated on a large scale. Having a well-validated test that has been tested against a gold standard (viral neutralization assays or another laboratory-based serological assay) is essential. The test's performance characteristics (sensitivity, specificity, positive and negative predictive values, cross-reaction to other coronaviruses) should be established using sera from patients infected with SARS-COV-2, other respiratory viruses, including seasonal coronaviruses, and healthy controls. The sera from patients should also reflect a range of infections, from asymptomatic to severe infections, and should be collected at different periods, from early infection to several weeks or months post-infection. In these various cases, it is possible that the immune response will be different, so a range of samples is needed to adequately characterise the conditions under which the test results are accurate.

Considerations relating to the application of rapid serological tests

Based on currently available information, international regulators and PHAC recommends that POC serological assays not be used for clinical testing in any capacity. In general, these antibody tests often do not become positive until a week or more after symptoms have started, and therefore are not suitable for acute diagnosis of COVID-19 at this time. As more information becomes available on test performance, and assays are validated against gold standard serological methods, the clinical application of POC assays will be re-evaluated. Molecular testing, such as real-time PCR, remains the primary test method for laboratory confirmation of COVID-19.

Importantly, there is still a lot of information not yet confirmed about the immune response generated by SARS-CoV-2 that limit the ability to rely on these tests.

- How soon antibodies are produced after infection?
  - Using a test before the antibodies have developed would result in a false negative result.
- How long after infection can antibodies be detected?
  - Using a test too late would result in a false negative result.
- How often does the test cross-react to detect antibodies to another virus?

        - A test that produces a cross-reaction is a false positive result. This could result in a return to work by a health care worker who has not developed antibodies to COVID-19.
    - Do mild infections, or asymptomatic infections result in detectable levels of antibodies?
        - Using serology to determine the level of past infections in a population will produce underestimates if asymptomatic infections do not produce measurable levels of antibodies.
    - Do antibodies protect individuals from second infections, or reduce the severity of those infections?
        - There are hypotheses that previous exposure to coronaviruses may exacerbate the reaction to a second infection, and that this may be why elderly people are so much more vulnerable to COVID-19 than younger people.
        - Korea and France has had patients be infected and become sick with COVID-19 a second time.
        - This is a very important consideration if using these tests to determine whether health care workers can return to work because they are considered 'immune'.

However, once the dynamics of the serological response in COVID-19 are better understood, serology can play an important role in the public health response. Provisions to ensure the capture of testing data for surveillance purposes and participation in external quality assessment to maintain high-quality testing will be key to making best use of this technology. Additionally, consideration will need to be given to the challenges of testing for antibodies in a large population of negative samples (it is expected that only a small percentage of the Canadian population has been infected with COVID-19). Under such circumstances, the rate of false positive results from the test may actually be higher than the rate of true negatives. Nonetheless, examples of potential uses include:

- Seroepidemiology, used to better understand the proportion undiagnosed in the population over time and to provide more accurate attack rates and mortality rates.
- Informing targeted diagnostic testing strategies, where priority would be given to populations/areas with no evidence of immunity.
- Detecting seroconversion in healthcare workers and other essential workers as a return-to-work measure from home isolation.
- Assessing sero-immunity in frontline workers.
- An adjunct to PCR for diagnostic testing in patients who are PCR-negative and in the late course of their illness to implement control measures and to effectively manage patients.
- Testing high risk populations exposed to SARS-CoV-2 to assess their risk of developing infection.
- Detecting seroconversion as a surrogate for effectiveness of control measures.
- Once a vaccine is available, it may be used to determine who should be prioritized for earlier vaccination.

These tests may have significant medical, public health, societal and economic implications. Some of these implications are highlighted below:

Medical	Individuals identified as having immunity to COVID-19 can donate plasma as a possible treatment for individuals who are critically ill from COVID-19. As well, these tests may identify healthcare workers who have recovered from initial infection and may be able to return to frontline health services
Public health	These tests may answer epidemiological questions about the spread of the virus and inform a vaccine strategy.
Societal	May inform decisions around which individuals can leave the home and be exempted from social distancing rules.
Economic	Information from these tests may play a role in identifying individuals who could return to work.