Serology testing for the presence of antibodies against COVID-19

28 April 2020 – prepared under urgency

Focusing specifically on antibody testing, this review builds on '[Testing for COVID-19 – the current state of laboratory tests and recommendations within the context of New Zealand's pandemic response](#)', a review led by Professor Michael Bunce, Chief Scientist at the Environmental Protection Authority (EPA) – Te Mana Rauhī Taiao, published on 30 March 2020.

Key messages

- Serology tests for the presence of antibodies in blood have the potential to reveal who has been exposed, and might now be immune, to COVID-19.

- As yet, there are no validated serology tests for COVID-19 antibodies that demonstrate high enough sensitivity and specificity to be used as the sole test for current or past COVID-19 infection, in any setting (laboratory, point of care, or home).

- The risk of using a bad test is worse than using no test at all, particularly in Aotearoa New Zealand right now because the lower the prevalence COVID-19 in the community, the greater the chance of amplifying incorrect results. Using tests with insufficient specificity will result in unacceptable levels of false positives, leading people to believe they have previously been infected with COVID-19 when they have not. Using tests with low sensitivity will result in unacceptable levels of false negatives, resulting in people thinking they have not yet been exposed or infected when they have.

- Antibodies develop at different times following infection with COVID-19, and often antibodies are not present when the patient is most infectious. A patient believing that a negative antibody test meant they were clear of disease could have disastrous consequences.

- We don't yet know whether a person with antibodies is actually immune to reinfection and for how long. Based on similar viruses, we presume that the majority of people will develop immunity following infection and it will last up to a year, but this is yet to be confirmed.

- Aotearoa New Zealand is using an elimination strategy, has had a low number of COVID-19 cases, and looks to be on the path to relax physical distancing measures and reopen the economy if this trajectory continues. As a result, we are not relying on population-level data about past infection to inform decisions, and approaches like the 'immunity passport' being proposed in other countries are less relevant to our current situation.

- Aotearoa New Zealand is in a position where there is no pressing need for wide-scale serology testing for COVID-19 so we can wait for reliable and validated tests to become available before applying serology to better understand the infection dynamics in our communities. This position could change quickly if there is a spike in local cases. Therefore, it is important to keep a close watching brief on the many serology tests in use or development, but hold off bulk purchasing for now. Efforts underway by labs in Aotearoa New Zealand to validate immunoassays should continue.

- Even when we have settled on a validated COVID-19 serology test, it should not replace the gold standard PCR tests for diagnosing COVID-19. Serology tests cannot pick up COVID-19 infection in the first week or so of infection because of the delay in antibody production. At most, these tests could be used as a backup diagnostic test when a suspected case who has been sick for several days tests negative by PCR.

Key resources

- Hundreds of serology tests are commercialised or in development and the number continues to grow. Up-to-date databases of serology tests are available at https://www.finddx.org/covid-19/pipeline/ and https://sph.nus.edu.sg/covid-19/research/
- Available performance data is collated at https://finddx.shinyapps.io/COVID19DxData/
- There are free online calculators that allow you to plug in different sensitivity and specificity values to see how these impact the probability that the disease is present when the test is positive.

Contents

Serology testing could play a key role in the COVID-19 pandemic

Upon infection with a virus, the body mounts an immune response which includes creating specific proteins (antibodies called IgG, IgM and IgA) that recognise and fight the virus's proteins (antigens). Rather than testing for the presence of the virus itself, serology tests for antibodies detect whether specific antibodies are present. The presence of these antibodies provides evidence that an infection has occurred.
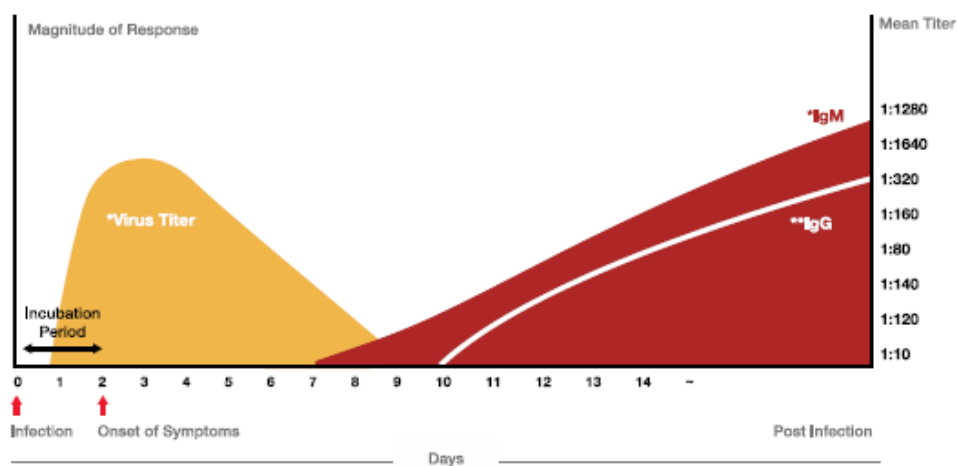

Figure 1: Virus infection and antibody response timeline sourced from the Royal College of Pathologists of Australasia. The delay in IgM and IgG until around 7 days after infection onset illustrates why this testing generates false negatives in the early stage of infection.

Depending on when and why serology testing is used it may be able to answer different questions.

Does this person *currently have* COVID-19?

Diagnosing current COVID-19 infections using serology testing is possible but limited in its application because antibodies only become detectable around seven days after infection (anywhere between 4–20 days). Consequently, serology tests only detect COVID-19 later in the course of infection, and would result in false negative results in the early days of infection when the patient is most infective. PCR testing remains the gold-standard diagnostic test for detecting those currently infected with COVID-19.

Serology testing may be used in diagnostics as a backup test for suspected cases who test negative with PCR (later in the course of their infection) or to detect positive cases who have had no or mild symptoms. For these cases, a low viral load may lead to a negative PCR result, or for an individual not to be tested at all, but the immune response could be detected via antibody testing. However, there are reports that up to 30% of infected patients have very low levels of COVID-19 antibodies, particularly those with mild or no symptoms, so the use of these tests for mild disease remains to be proven.

Has this person *already had* COVID-19?

Because antibodies are still present after the virus has run its course, serology testing may be used for retrospective diagnosis of COVID-19 infection. This approach is likely to be required in areas where PCR testing capacity has been exceeded or the healthcare system has been overwhelmed so suspected cases were not able to be tested. Another benefit of retrospective testing is that it may be

able to pick up people who had no or mild symptoms and didn't realise they were infected, helping to paint a clearer picture of population-level infection dynamics. The effectiveness of this approach in understanding past infections depends on the sensitivity and specificity of the individual test.

Antibody tests cannot rule out that a person is no longer infectious.

Is this person *immune* to COVID-19?

A serology test provides evidence that antibodies are present but as so little is known about COVID-19 immunity (we don't yet know the level of antibodies required for immunity or how long that immunity will last)[1] it doesn't tell you whether a person is immune to reinfection. Based on similar viruses, experts suggest that the majority of people will develop immunity following infection and it will last months or years, but exactly how long is unclear.[2] Further research is needed to understand the COVID-19 immune response. If research confirms that antibodies confer immunity and the level at which they do, a serology test could be used to provide evidence of immunity. Some countries are looking at using these tests to provide "immunity passports" that prove a person is able to return to a country or workplace because they are immune to COVID-19. But this based on assumptions about immunity that are yet to be confirmed and so would be extremely risky.

How many people in the *population* have had COVID-19 and are immune to it?

Using serology tests at a population-level (serosurveys) can provide data on the level of COVID-19 exposure in a population by determining whether someone has previously been infected. The same approach for population-level analysis of immunity would only be valid if we know that the presence of antibodies means a person is immune to reinfection – this has not been confirmed. Representative sampling is necessary for serosurveys and adjustments to the population-level estimates can be made to account for rates of false positive/negative tests expected if the assay's sensitivity and specificity performance has been verified beforehand. Some countries are exploring the use of serosurveys to determine the level of immunity among the population to, for example, determine if a level of herd immunity has been reached (i.e. enough of the population is immune to reduce spread of infection throughout the rest of the population).

Most available serology tests have not undergone rigorous testing to ensure they are reliable

The rush to market for COVID-19 serology tests has resulted in little to no validation occurring before these tests have been introduced. There can be huge variability in the repeatability and reproducibility of assays due to the type of test, the manufacturing process and how the test is run. There are currently no agreed standards or controls available for serological testing for COVID-19 antibodies.

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[1] Bao et al., "Reinfection Could Not Occur in Sars-Cov-2 Infected Rhesus Macaques," *bioRxiv* (2020); Wu et al., "Neutralizing Antibody Responses to Sars-Cov-2 in a Covid-19 Recovered Patient Cohort and Their Implications," (2020); Wang et al., "Long-Term Coexistence of Severe Acute Respiratory Syndrome Coronavirus 2 (Sars-Cov-2) with Antibody Response in Coronavirus Disease 2019 (Covid-19) Patients," *medRxiv* (2020)
[2] Wu et al., "Duration of Antibody Responses after Severe Acute Respiratory Syndrome," *Emerging infectious diseases* 13, no. 10 (2007)

The potential for false results generated by low-quality assays to inform decisions during this pandemic is concerning. Even the tests that appear to have been validated need to be considered with caution given issues around sample size and population-level representation. A false sense of security may arise from tests being approved by the FDA (US) and TGA (Australia), but these agencies are not applying their normal stringency in evaluating tests prior to approval. Given the lack of validation occurring prior to tests being put on the market, it is essential that any serology test is properly evaluated by those purchasing the tests before they are used.

Two key measures that need to be scrutinised during validation are sensitivity and specificity.

Sensitivity: this relates to how accurate the test will be at giving a positive result when a person is a true positive (e.g. they had a confirmed COVID-19 diagnosis by PCR). If a test has low sensitivity, it will under-count the number of positives. The positives that are missed are called "false negatives". The higher the test's sensitivity, the better.

For COVID-19, the antibody response may differ by age group, severity of the infection, or pre-existing conditions that compromise immunity. A test needs to be able to pick up low levels of antibodies to have high sensitivity. Depending on the population tested during validation, the sensitivity of the test could be inaccurate. If only individuals with severe or prolonged infections were included in validation studies, the sensitivity would be overstated and the test may not actually pick up those in early stages of infection or with more mild symptoms.

Specificity: this relates to how likely the test is to only pick up a positive result for the virus of interest and no other similar infectious agents. If a test has low specificity, it could generate a positive result for a person that hasn't had a COVID-19 infection. These incorrect positive results are called "false positives".

The virus that causes COVID-19 (SARS-CoV-2) is closely related some viruses that cause the common cold, and if the test is not specific enough to SARS-CoV-2 it may give false positive results for people who have only had a cold, or other similar infections. Whether or not this issue is picked up during validation depends on the population used in the validation study. If none of the people in the study have recently had the common cold or other respiratory viruses (which are seasonal) we wouldn't know how specific the test really is.

Point of care tests are generating the most interest, but lab-based serology testing will be more reliable

There are a number of different techniques used for serology testing (details available in the appendix). These tests can either be performed in the lab or at 'point of care' (POC) – e.g. at a testing centre or a GP's clinic. Tests that can provide results rapidly ('rapid testing platforms') are suitable for use at POC because the patient can receive the result immediately, rather than having their sample taken, sent to the lab, and results returned days later. Some serology techniques are more amendable to rapid testing platforms than others. In particular, the lateral flow immunoassay (LFIA) technique is well-suited to be developed as rapid test to be used at POC (or in the home setting) because they are cheap and fast to manufacture, and allow for fast test development and interpretation of results.

Though rapid testing platforms that can be performed at POC have potential, lab-based tests performed by experienced professionals have inherent quality checks and validation can be carried out in these settings. Preliminary studies also suggest better sensitivity and reliability of these tests (pre-print only, not yet peer reviewed).[3] These test types include ELISA and Neutralisation Assays, both of which can be internally designed and validated in the lab. Commercial EIA ELISA kits are also available.

Figure 2 Example of a POC test for COVID-19 antibodies that shows a positive test result

Due to urgency in the COVID-19 response, some health authorities such as the [FDA (US)](#) and [TGA (Australia)](#) are granting emergency urgent provisions for the use of tests for COVID-19 without the usual levels of scrutiny. So far, all emergency approved serology tests are LFIAs. Studies evaluating the sensitivity and specificity of some of these LFIA tests highlight variable performance (pre-prints only, not yet peer reviewed).[4] Despite appearing to give a clear visual reading of a positive or negative result (akin to a pregnancy test, see Figure 2), LFIA tests require a level of scrutiny to determine that the test is working properly (i.e. the positive or negative control readings are correct) and to interpret results (e.g. if the line is faint). New batches of test kits may also need to be run with positive controls to ensure they are generating accurate results. For these reasons, when validated LFIA tests for COVID-19 are available, their use should be limited (at least initially) to use by health professionals and not home use.

Until validated POC tests are available, it is advisable that serological tests are requested by clinicians, performed in accredited diagnostic laboratories and interpreted by clinical microbiologists.

The role of serology testing in Aotearoa New Zealand

On April 22, [Medsafe announced a ban on the importation and sale of all POC COVID-19 test kits, unless they gain approval](#), using the provisions in [section 37 of the Medicines Act 1981](#). No POC test for COVID-19 has so far been approved.

The New Zealand Microbiology Network released a [position statement on Rapid Diagnostic Tests for SARS-CoV-2](#), highlighting limitations and supporting further efforts to research and validate testing options.

Work is currently underway to build up capacity and capability for lab-based enzyme linked immunoassays (EIAs) for COVID-19 serology in Aotearoa New Zealand.

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[3] Lassaunière et al., "Evaluation of Nine Commercial Sars-Cov-2 Immunoassays," *medRxiv* (2020); Adams et al., "Evaluation of Antibody Testing for Sars-Cov-2 Using Elisa and Lateral Flow Immunoassays," *medRxiv* (2020)

[4] Lassaunière et al., "Evaluation of Nine Commercial Sars-Cov-2 Immunoassays,"; Adams et al., "Evaluation of Antibody Testing for Sars-Cov-2 Using Elisa and Lateral Flow Immunoassays,"

Serology testing in action overseas

Due to the urgency and scale of the COVID-19 pandemic, several countries have begun to implement serology testing as part of their response, despite the limitations discussed above. As at April 8, the WHO advised against using new POC serology tests in clinical decision making because of reliability issues and the insufficient evidence that presence of antibodies correlates to immunity to reinfection. The WHO recognises the potential for these tests to make a huge difference in the pandemic and encourages further research, including through the Solidarity II study it is coordinating.

- **United States:** Various counties and states are undertaking serology testing using different tests and methods. Some examples are highlighted below.
    - The CDC is doing 'broad-based surveillance and research' with serology testing, starting in Washington State and NYC. Results are not yet published and it is unclear which specific tests are used.
    - Santa Clara study by Stanford University – pre-print not yet peer reviewed.[5] This study used a COVID-19 IgG and IgM rapid test manufactured by Hangzhou Biotest Biotech, Co., Ltd. and distributed by Premier Biotech. They found one in 66 people tested were positive for antibodies against SARS-CoV-2, extrapolating this finding to estimate that 50-85x more people had been infected than official reports. This finding has garnered a lot of attention, but there are many limitations including reliability of the test; the high likelihood that based on company performance data, the majority of positive results could be false positives; sampling bias due to Facebook recruitment; low numbers tested for 'in-house' validation; questions around the weighting methodology used to adjust results.
- **Australia:** Numerous POC serology tests have received expedited approval by the TGA and the Peter Doherty Institute for Infection and Immunity has been funded to undertake a post-market assessment of new COVID-19 rapid tests
    - The Royal College of Pathologists of Australia (RCPA) released a position statement strongly opposing the introduction of serology tests for COVID-19 diagnostics
- **United Kingdom:** Failure of validation studies[6] to confirm tests are of high enough sensitivity and specificity for national roll-out has seen over 4 million POC serology tests go unused.
- **Singapore:** Singapore reported the first use of serology testing to retrospectively diagnose two asymptomatic COVID-19 patients, establishing the missing link between clusters.
- **Germany:** Three large serosurveys are planned, but not yet underway.
    - Preliminary results from a serosurvey (and questionnaire) that so far tested 500 people in a village of more than 12,000 found that 14% had been infected with SARS-CoV-2. No details were given in the report (released in German) on testing methodology or questionnaire details, though a test specificity of >99% for the IgG test is reported.
- **Croatia:** Authorities are undertaking a serological survey but results are not yet available.
- **Italy:** Serology testing in Robbio county is underway. Of the 6000 residents, 1,300 people have been tested so far, with 11.5% having positive tests. Details of the tests used and validation data are not yet available.

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[5] Bendavid et al., "Covid-19 Antibody Seroprevalence in Santa Clara County, California," *medRxiv* (2020)
[6] Adams et al., "Evaluation of Antibody Testing for Sars-Cov-2 Using Elisa and Lateral Flow Immunoassays,"

- **Scotland:** A pre-print (not yet peer reviewed)[7] of a COVID-19 serology study on recent blood donors was undertaken using a neutralisation assay and verified by ELISA (lab-based tests not POC). Samples from an earlier date had no positive tests, but 5 out of 500 of those from a later date were positive (1% of sample). This study incorporated good controls but was limited in selection bias of blood donor cases not being fully representative of population (e.g. age/weight exclusions, exclusions of people with a history of drug use).

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[7] Thompson et al., "Neutralising Antibodies to Sars Coronavirus 2 in Scottish Blood Donors - a Pilot Study of the Value of Serology to Determine Population Exposure," *medRxiv* (2020)

Acknowledgements

Glossary

- Antibody – A protein made by your body to combat infection by virus. Antibodies to COVID-19 could protect the body from re-infection as they can neutralise the virus.
- Antibody test: Is a test to see if a patient has generated antibodies (see IgG and IgM) to the COVID-19 virus.
- Antigen test: Is a test for the SARS-CoV-2 virus that detects surface proteins of the virus rather than the viral RNA (see PCR).
- B-cells: Cells in the immune system that are responsible for antibody production.
- COVID-19: The name given to the disease pandemic caused by the SARS-CoV-2 virus which arose in the Wuhan Provence in China in late 2019.
- ELISA: Enzyme-linked immunosorbent assay. In the context of COVID-19 this is a test conducted inside a diagnostic laboratory to detect antibodies (see IgG and IgM). It could be used as a barometer of immune response to the virus.
- False positive: When a test for COVID-19 is positive yet the person has not been infected with COVID-19.
- False negative: When a test for COVID-19 is negative yet the person is or has been infected with COVID-19.
- IgG: A type of antibody that is typically generated late in the COVID-19 infection
- IgM: A type of antibody that is typically generated early in the COVID-19 infection
- Lateral flow: A type of 'dipstick' point-of-care test akin to a pregnancy test, where body fluids are deposited on the device, which will (in 10-20 mins) reveal if antigen or antibodies to the virus are present.
- Monoclonal antibody: This is a single 'type' of antibody that can detect Virus proteins. Making monoclonal antibodies is complex and finding one that is precise and can be produced at scale is time consuming. Monoclonal antibodies will ultimately be used as basis for point-of-care antigen tests.
- PCR: 'Polymerase Chain Reaction'; a test by which RNA (or DNA) is photocopied. This is the core test for COVID-19 virus as it is very sensitive and specific.
- POC: 'Point-Of-Care' a test that is typically a 'yes/no' answer that is conducted rapidly at the patient's bedside or their home.
- SARS-CoV-2: The virus that is responsible for the COVID-19 epidemic.
- Serological: A term used to describe how our body fluids respond to the virus, for example the formation of antibodies.
- Validation: The process by which a test for COVID-19 is assessed to see how reliable it is. Test validation takes many forms but centres around how well a test performs in terms of sensitivity and precision. When products are rushed to market validation may be poor.

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Adams, Anand, Andersson, Auckland, Baillie, Barnes, Bell*, et al.* "Evaluation of Antibody Testing for Sars-Cov-2 Using Elisa and Lateral Flow Immunoassays". *medRxiv* (2020)

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Wu, Wang, Liu, Wang, Chen, Xia, Ling*, et al.* "Neutralizing Antibody Responses to Sars-Cov-2 in a Covid-19 Recovered Patient Cohort and Their Implications". (2020)

Appendix

Details of the different techniques that can detect antibodies via serological testing.

Lateral flow immunoassay

This qualitative (positive or negative) assay is generally small, portable, and able to be used at point of care (POC). These tests may use blood/plasma samples from a vein (in some cases a finger prick), or saliva samples. The test shows the user coloured lines to indicate positive or negative results. Importantly, the amount of antibody in the sample must be high enough for the colour change to be definitive, relating to the test's sensitivity. In the context of COVID-19, these tests most frequently test for patient antibodies (IgG and IgM), however they can be designed to detect viral antigen.
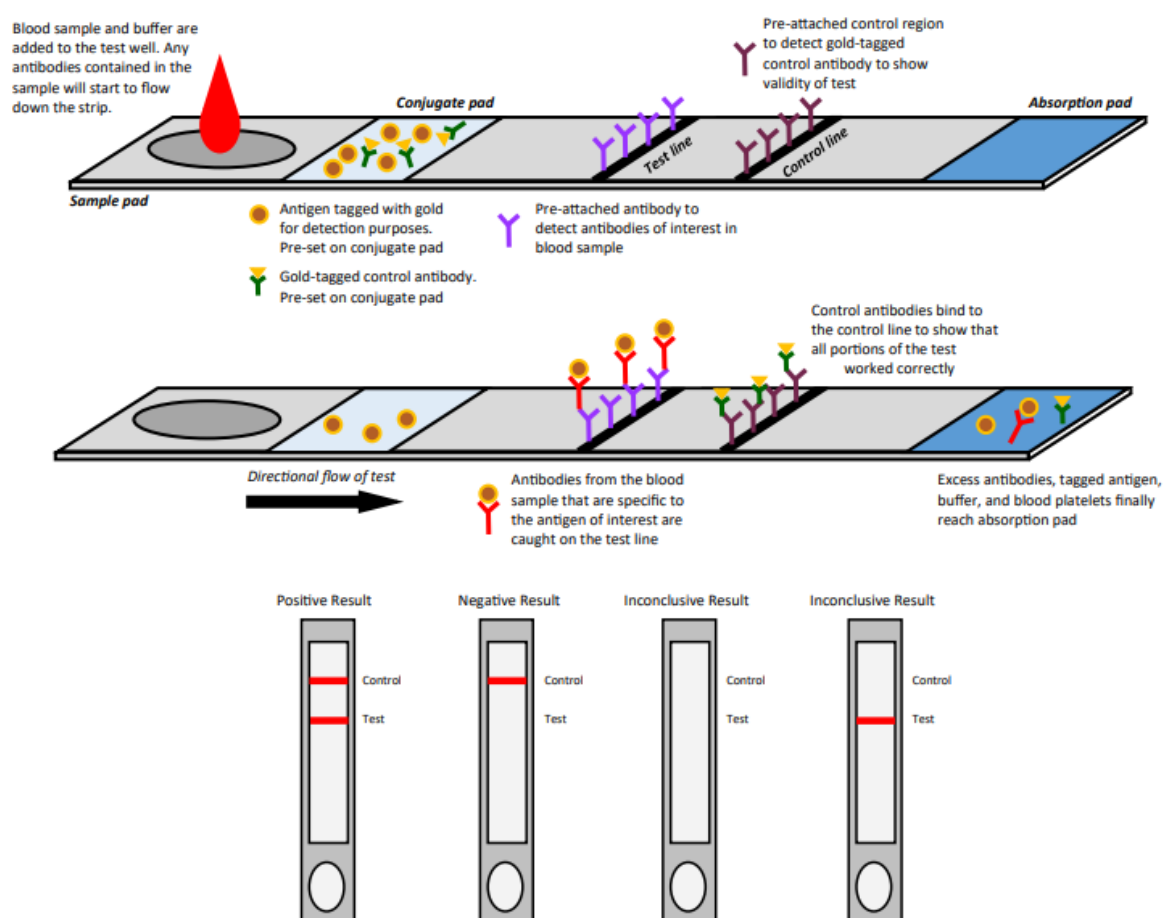

Figure 4 Lateral flow POC assay basics. Source: http://www.centerforhealthsecurity.org/resources/COVID-19/serology/Serology-based-tests-for-COVID-19.html

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a preferred antibody assay for use in a diagnostic lab. It typically comes as a commercial kit with reagents specific to the target organism. This test can be qualitative or quantitative and is generally a lab-based test. It is likely that the qualitative yes/no results will be highly sensitive and selective. The quantitative analysis may not be so accurate. Samples used usually include whole blood, plasma, or serum. For COVID-19, these tests can evaluate the presence and amount of patient antibodies (IgG and IgM).

The ELISA test (Figure 3) relies on a 96 well, single-use plastic plate and a benchtop spectrometer and involves a laboratory protocol that takes 1-5 hours (depending on incubation times required for optimal binding and colour development). When used correctly, these tests are considered reliable for qualitative analysis. Accuracy of quantitation varies depending on many factors including (but not limited to):

- Concentration and orientation of antigen binding to the surface of the plastic plates.
- Individual patient antibodies binding ability to the selected antigen.
- Washing protocols used.
- Sample variance – blood samples can have proteins that stick to and foul the plastic plates making the quantification result less accurate.
- Accuracy of concentration of the visualising reagent (added by lab technician, this is a human error element).

Some drawbacks of this technique include the need for specific lab equipment, use of reagents (that could become limited if supply chain issues arise), length of time to perform the test and get the result, and need for skilled technicians.
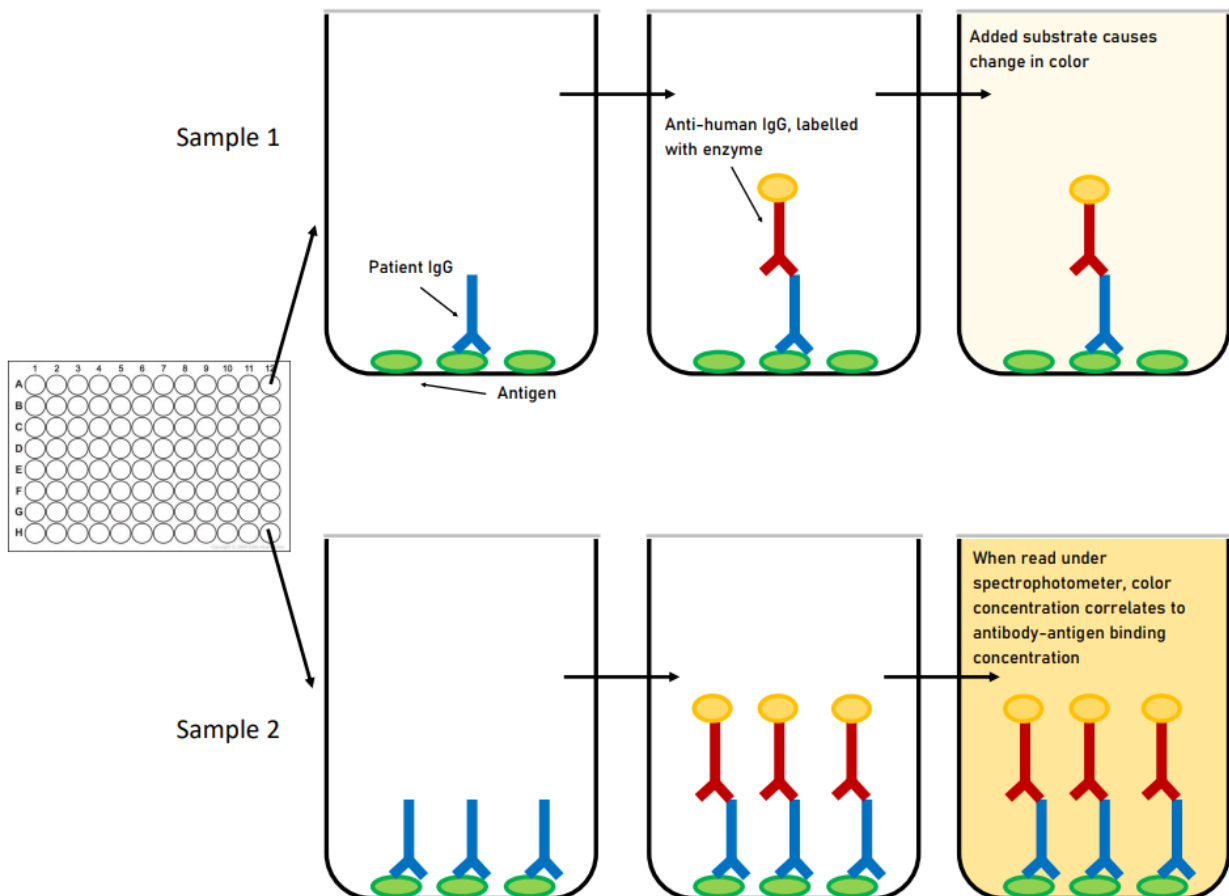

Figure 3 Basic concept of an ELISA assay

Fluorescence based techniques for antibody detection require a fluorescence microscope to see the result, making these test methods inappropriate for POC and at home testing.

DFA tests use fluorescently labelled antibodies that specifically bind to the viral proteins that are present in the patient sample. This illuminate the virus so it can be visualised using a fluorescent microscope. This technique can be used to identify if a patient is infected. A secondary way this technique can be applied is by using cell flow cytometry, an automated cell sorter that can distinguish between cells that have a fluorescent label and those that do not.

IFA tests are used to look for antibodies in patient serum and they work very much like ELISA assays except that there is no need for a visualising reagent. The fluorescent label is detected using a fluorescent microscope.

Some drawbacks of these techniques is that they are impacted by specimen quality and collection method, do not offer immediate results, and require skilled technicians and lab equipment.
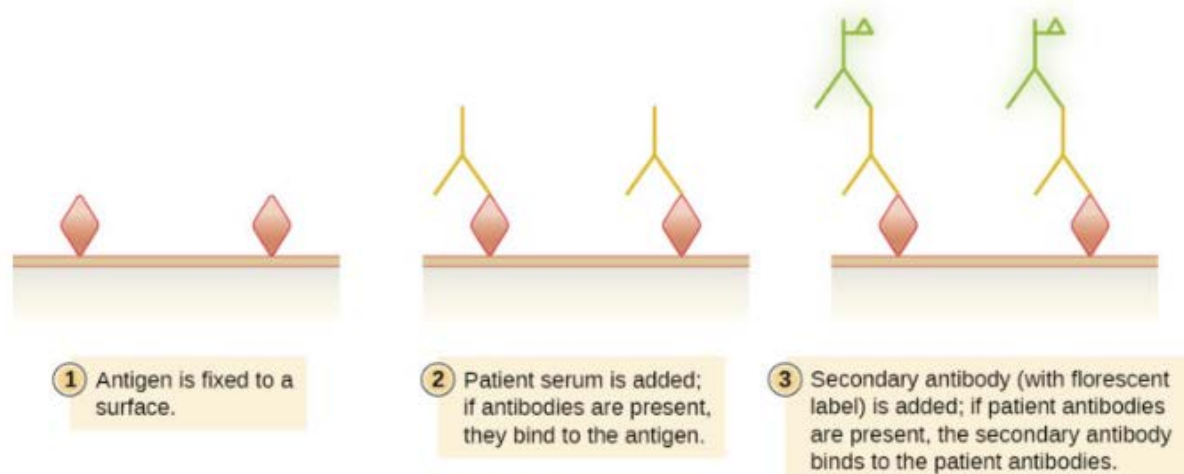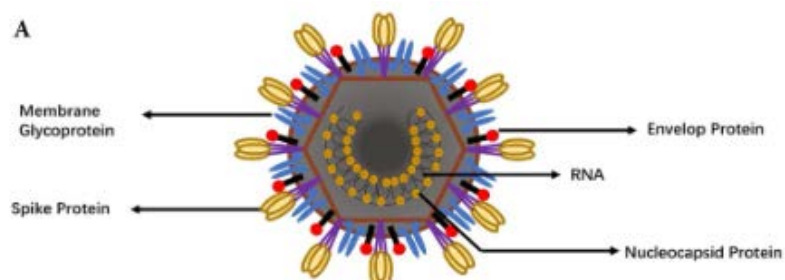

Figure 3 Illustration of indirect IFA assay principles

Neutralisation assay

A subset of antibodies that are made in the body, called neutralising antibodies (Nabs), reduce virus infectivity by binding to specific protein on the outside of the virus that are used to gain entry into the human cells. Nabs block the human cell target of the virus.
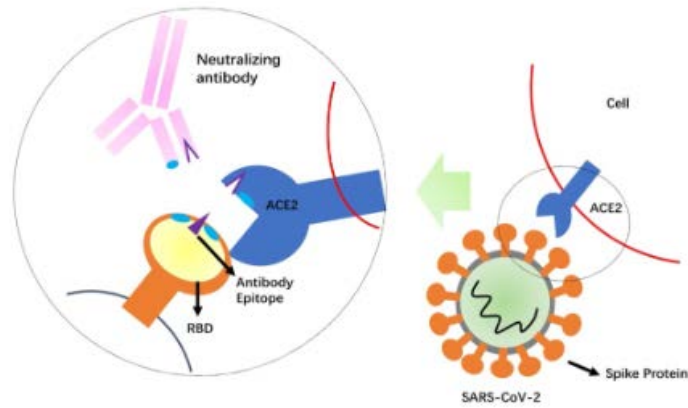
Figure 4 (A) Viral proteins of SARS-CoV-2, (B) Illustration of Nabs in competition with human cell receptors for binding to S protein of SARS-CoV-2.

The Receptor Binding Domain (RBD) within the Spike protein (S protein) has been identified as a key neutralisation target for SARS-CoV-2 Nabs.

Neutralisation assays can determine if a patient has antibodies that are functional and effective against the virus, even if they have already cleared the infection (immunity). These tests require whole blood, serum, or plasma samples from the patient and depend on cell culture, a lab-based method of culturing cells that allow SARS-CoV-2 growth. When virus and cells are grown with decreasing concentrations of patient antibodies, researchers can visualize and quantify how many antibodies in the patient serum are NAbs.