Rapid antigen test for SARS-CoV-2

Technical White Paper

elg medical

1. Introduction

As of the 1st October 2020, there are over 33 million confirmed cases of COVID-19 worldwide, with over 1 million deaths (https://covid19.who.int/). The WHO, as well as many governments, have emphasised the necessity of increased testing as a means of screening the disease, understanding its epidemiology, and assessing the safety of the public to return to work. RT-qPCR is currently the most common method of detecting the virus, however, there are drawbacks to this testing method.

Antibody tests have an important place in the testing protocols for COVID-19, to increase accuracy in testing alongside PCR for widening the detection window for the test and allowing better understanding of the spread of infection through the wider population. We have developed a rapid lateral flow immunoassay (LFIA) test for the qualitative detection of two antibodies specific to SARS-CoV-2. The advantages of this test are low sample volume, multiple sample types, speed of test, ease of use, cost-effectiveness and easy to read results.

Recently, an antigen test was also developed, which detects specific viral proteins. The advantages of this test are like those for the antibody test, as detailed above. As the antigen is detectable earlier than antibodies, combining the two tests will lead to effective diagnosis, a larger detection window, and an indication of previous infection. This report summarises the workings of the antigen test, comparisons of antigen and antibody tests and how they can be combined for effective diagnosis of COVID-19.

2. How The Antigen Test Works

The test consists of a lateral flow immunoassay device. The sample is mixed in a buffer solution, which is added to the test strip. The buffer solution allows the sample to flow along the test strip, where it interacts with antibodies specific for the antigen.

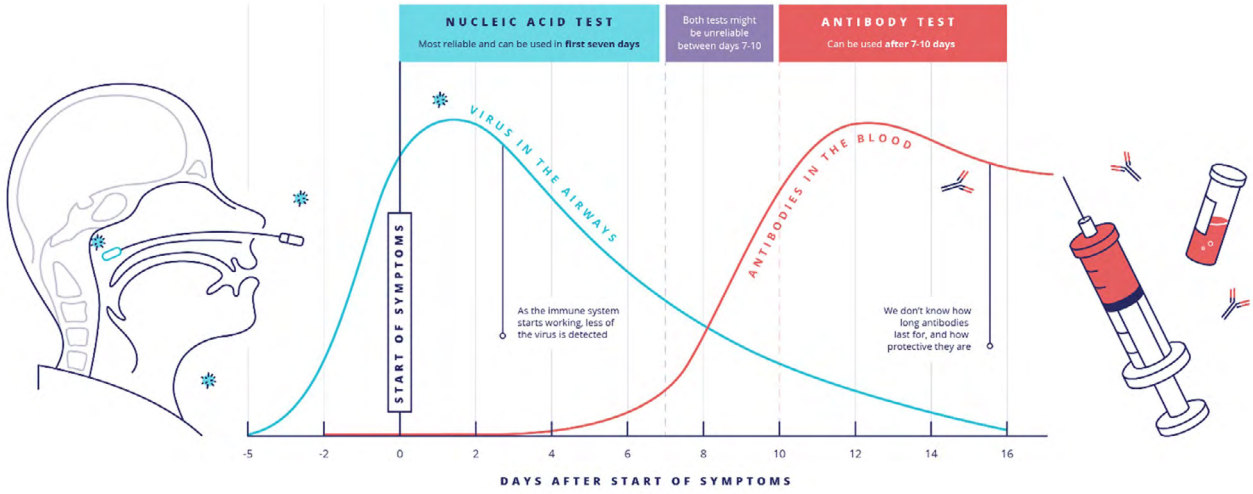
If the COVID-19 antigens are present in the sample, the antibodies will bind to them, after which they will flow along the strip and bind to a second set of antibodies which are fixed at defined locations on the test strip. If the antigen is not present in the sample, then a reaction will occur at the Control Line (C), but no reaction will occur at the Test Line (T). The test results are ready to be interpreted.

Lines detected at both the Test Line (T) and Control Line (C) indicate a positive result, whereas a line detected at the control line only indicates a negative result. If a control line is not detected, the test is void and should be discarded. This process takes less than 15 minutes, making the entire test procedure under 20 minutes. Additionally, multiple test strips can be tested back to back, bringing further efficiency to the process.

3. Antigen vs Antibody

An antigen is the entity causing the illness. In the case of COVID-19, the antigen is the virus itself, or more specifically the proteins of the virus. Antibodies are immune proteins produced by the body in response to the antigen, and act to 'flag' the antigen as harmful so that the rest of the immune system can clear it.

The window of detection given to COVID-19 antigen and the antibody response is different. For the antigen, the detection window is similar to conventional nucleic acid tests (such as qPCR). Detection is possible as early as 2-3 days before symptoms develop and peak the day after symptoms appear. Antigen tests become less reliable over time, as the immune system begins to clear the virus and the virus begins to descend into the lower airways (rendering throat swabs less effective). Our antibody test has been shown to detect antibodies as early as 3 days after symptoms develop and reach its peak sensitivity after 14 days post symptoms. The below illustrates the timeline of both antigen and antibodies.



Source: UK Research and Innovation (https://coronavirusexplained.ukri.org/en/article/vdt0006/).

4. Cycle Threshold (Ct) Values and RT-qPCR

The current 'gold standard' in clinical diagnostics for COVID-19 is reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Briefly, this method takes the viral RNA, reverse transcribes it into DNA and then amplifies the DNA copies until a fluorescent marker is detected. The presence or absence of a fluorescence signal gives a positive or negative result, respectively.

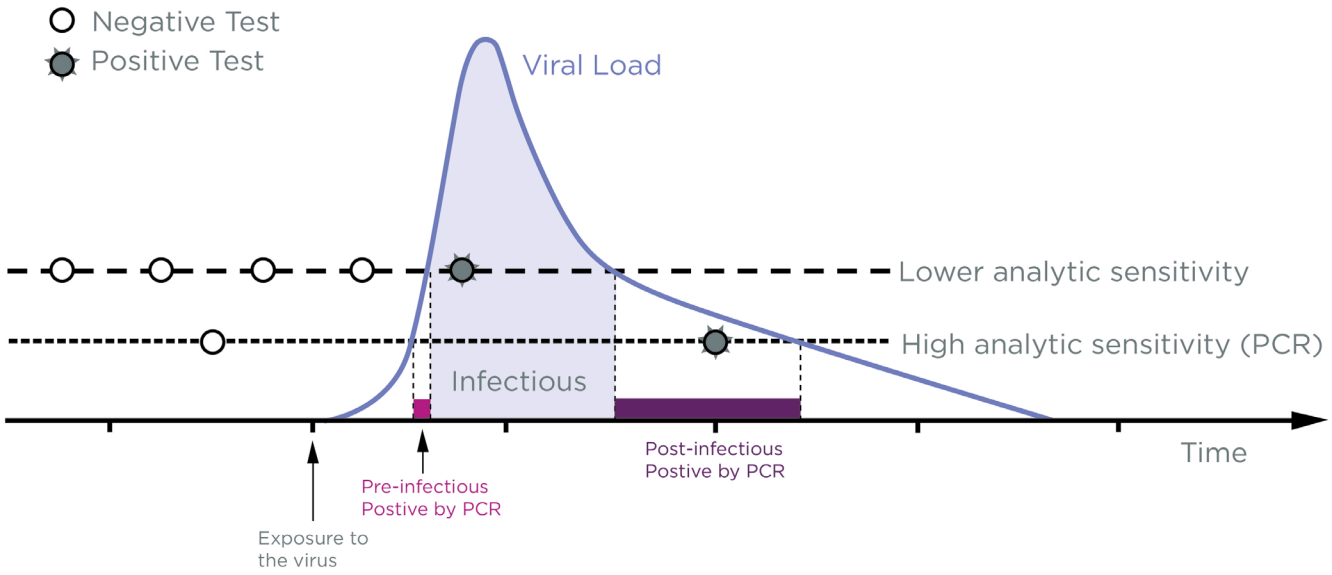
As RT-qPCR continually increases the number of DNA copies, in a positive sample, eventually there will be enough copies to give a fluorescence signal. Hence many PCR systems employ a cut-off value, based on the cycle threshold (Ct). The Ct is the number of PCR cycles required to give a positive result. In general, a lower Ct value means the sample had a high number of viral RNA copies (therefore fewer PCR cycles were required to give a fluorescence signal). The cut off Ct value is the number of cycles above which the viral RNA copies are too low to be classified as a positive COVID case. Researchers also use this cut-off value as an indicator of whether a person can spread the virus to another person. Most diagnostic PCR systems use a cut-off Ct value of 30. This typically equates to viral RNA loads of <100 copies per mL of sample, making the PCR test extremely sensitive.

5. Relationship Between Ct Values, Viral Load, And Infectivity

With enough cycles, PCR is able to detect very low viral loads, well below the level that would cause symptoms or infection spread. However, depending on the defined Ct cut-off, some of these cases may be missed at the early stage. PCR is suitable for diagnosing pre or asymptomatic COVID cases, however, it requires trained professionals to carry out the analysis and takes time to obtain a result. Hence, it is imperative to combine laboratory diagnostic procedures with rapid, inexpensive testing for the purposes of mass testing for COVID-19. Using combination testing methods is arguably the best method of detecting COVID-19, reducing further contagion.

6. Antigen Test Timeline

The figure below shows how the concentration of antigen changes over time. As shown, the viral load increases rapidly after exposure, before decreasing steadily. In testing for COVID-19, there has been much debate about sensitive laboratory screening versus rapid testing devices. This was recently analysed (Larremore et al. (2020), who argued that test sensitivity is secondary to test frequency in mass screening for the SARS-CoV-2 virus. There are several reasons for this; firstly, the viral kinetics means that the concentration of virus in the airways initially remains low, then rises rapidly before slowly tailing off. Repeat testing means that the virus can be detected at the earliest stage, as its levels rise above the detectable threshold of the test. However, using a highly sensitive test once is only effective if used at the optimum time and can be missed if used too early or late. Secondly, even if a rapid test has a low sensitivity, this can be improved by repeating at defined time intervals. For example, if a rapid test has a sensitivity of 80%, by repeating three times, the sensitivity increases to 99%. Therefore, testing repeatedly is hugely beneficial in detecting COVID-19, rather than single testing using highly sensitive equipment. Furthermore, combining different rapid screening devices, such as antigen and antibody tests, ensures that the maximum sensitivity can be achieved at the earliest time point.



7. Combination Testing

As illustrated in the figure above, antigens can be detected at the onset of symptoms (day 0 to 10) and antibodies can be detected later (day 3 onwards). At this stage it is unknown just how long antibodies remain in the body and what levels are required for immunity. However, the illustration shows that effectively combining antigen and antibody testing is extremely useful in maximising test sensitivity and covering the entire period of the infection in an individual. Therefore, the combination of rapid antigen and antibody tests provides an alternative to PCR for mass screening for COVID-19, especially in the workplace and in wider society. Effective antibody testing also allows for better understanding of the stage of infection and the longevity of COVID-19 antibodies.

8. Conclusion

Overall, the antigen test is an invaluable tool for early detection of the SARS-CoV-2 virus in the upper airway. The antibody test is an extremely sensitive and accurate method of identifying previous SARS-CoV-2 infection and determining immunity after recovery. As the two have differing detection windows, combining them can give a clear picture of an individual's exposure, infection, recovery, and long term immunity to the virus. Due to their high sensitivity, ease of use and quick test time, the two tests are suitable for mass screening of patients in healthcare settings and the wider population, such as the workplace, society, and in national and international travel.

9. References

1. Tom MR and Mina MJ (2020). To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value. Infectious Disease Society of America. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7314112/pdf/ciaa619.pdf)

2. Larremore DB et al. (2020). Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. (https://doi.org/10.1101/2020.06.22.20136309, https://www.medrxiv.org/content/10.1101/2020.06.22.20136309v2)

info@elgmedical.co.uk
+44 (0)2392 534 930
www.elgmedical.co.uk
ELG Medical, Brockhurst Industrial Estate, Gunners Way, Gosport, PO12 4DR, United Kingdom

elg medical