

Guest Author: Helen Whitby, Technical Specialist

Coronaviruses (like SARS-CoV-2) are enveloped viruses which possess a unique replication strategy with distinctive biological characteristics. These viruses are spherical in shape with small protruding spikes which give them the ability to attach to human cells. Once attached to a cell they undergo a structural change which facilitates fusion of the virus to the cell membrane and allows them to enter the host cell. The Coronavirus entry is mediated by the viral spike glycoprotein, which is a 180kDa glycoprotein and this is one of three major structural proteins which form a coronavirus.

The glycoprotein virions which form the viral spike have been characterized using size exclusion chromatography following solubilization with Triton-X; a non-ionic surfactant typically used for membrane solubilization. This 180kDa glycoprotein is then cleaved with trypsin into two 90kDa species and it is the analysis of this E2 subunit which is of major interest to scientists. It is thought to be an important determinant of the virulence of the coronavirus and whether the spread from cell to cell occurs without showing host immune response.¹

A lot of media attention in recent days has focused on how the testing for the SARS-CoV-2 strain of the coronavirus is done. There are currently two possible tests available; one using blood samples which look for specific antibodies and the second is using a throat swab and a different test known as polymerase chain reaction testing or PCR. PCR tests work by rapidly synthesising many copies of a specific part of the genetic sequence of interest; enough to

allow it to be studied at a greater depth. Some people refer to it as amplification.

SARS-CoV-2 contains around 30,000 nucleotides but this test targets around 100 of these which are specific to the SARS-CoV-2 genome. A section of the genetic code of the virus containing these nucleotides is extracted and reacted in a process known as thermal cycling. The first step of amplification involves denaturing of the genetic material which is then passed forward to the second stage referred to as annealing the reaction temperature is lowered and primers included in the reaction mixture adhere to the RNA strands and begin the replication process. Replication is extended using polymerases which facilitate the synthesis of a new strand of genetic code by adding free nucleotides from the reaction mixture to the strand in the 5'-3' direction. This process is repeated until the desired amplification is achieved.

The high sensitivity of PCR permits virus detection soon after infection and even before the onset of disease. Real Time PCR is currently being used for the rapid testing of sputum samples for the rapid testing of patients for SARS-CoV-2 however there are still a number of drawbacks.

All enzymes are prone to error and nucleic acid polymerases are no exception. Also a limiting factor with PCR is that even the smallest amount of contaminating genetic material can be amplified which can lead to misleading results. In a fast-moving clinical emergency environment this is made all the harder to control.²

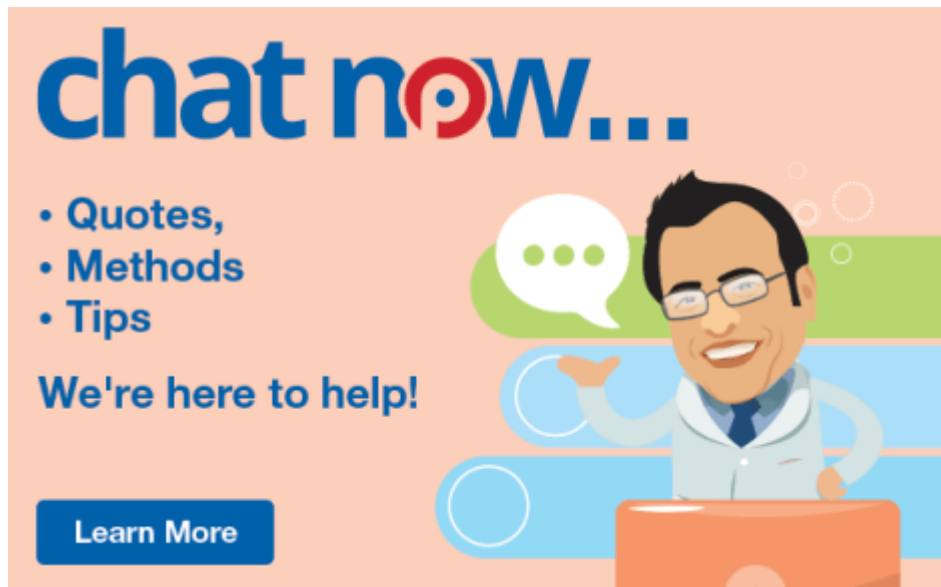
In conjunction with PCR tests clinicians are also looking at serology testing which can detect previous infection through an immune response to the SARS-CoV-2. Antibodies are produced in the body's immune response to attack by the virus during infection and can be detected by enzyme-linked immunosorbent assay or ELISA which is screening test used to detect the presence of a specific antibody which binds to a viral protein. Once an immune response is detected by ELISA, micro neutralisation is used to determine the presence of neutralising antibodies in a patient. If a positive is found in both these tests a patient is reported as testing positive for the SARS-CoV-2 virus.

Although highly specific the major draw back with this form of testing is the length of time it takes with most tests requiring 5-7 days to process.

A serological test can detect antibodies even if a patient has recovered and may help us learn more about the rate and source of infection; a PCR test however can only detect the virus only if the person is currently sick (infected?). However, both tests have the potential to miss cases if samples are taken too early, when the viral load is too low or if the person's body hasn't produced antibodies against the virus yet.

If you have any questions regarding this article or are looking for technical assistance of any kind, reach out to our technical specialist nearly 24/7 through Chat Now. You might even get to chat with Helen herself!

Chat Now: www.phenomenex.com/chat



Resources:

1. <https://www.biorxiv.org/content/10.1101/2020.03.16.994152v1.full.pdf>
2. Schochetman, Gerald; Ou, Chin-Yih; Jones, Wanda K. (1988). "Polymerase Chain Reaction". *The Journal of Infectious Diseases*. **158**(6): 1154-1157. doi:10.1093/infdis/158.6.1154. JSTOR 30137034

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