SARS-CoV-2 Testing Update

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The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and COVID-19 disease continues to spread throughout the world with millions of individuals infected and probably many more infections will be recorded in the coming months globally as well as nationally.

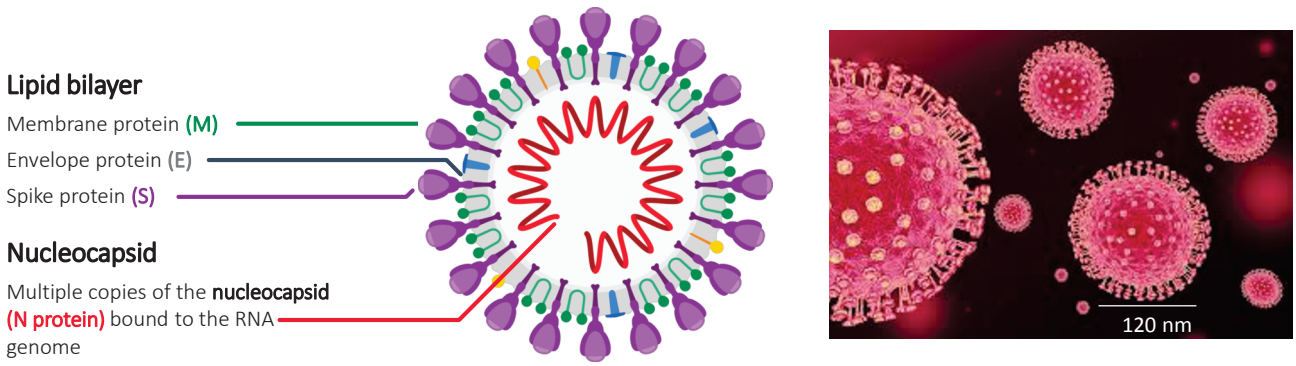
Laboratory tests play a critical role in this pandemic. Tests have been used for diagnosis of COVID-19 disease in symptomatic individuals, for public health interventions and containment including case identification and contact tracing. With the advancing epidemic laboratory tests, designed as diagnostic tests have increasingly been applied to the screening of asymptomatic individuals in the workplace, entering hospital facilities for medical care and procedures or those in long term care or correctional facilities. PCR testing resources and the less than ideal way this test has been used necessitated looking for alternative testing platforms that could be used. SARS-CoV-2 serological testing has been developed as an adjunct to the existing standard PCR test for diagnosis as well as assisting with testing of asymptomatic individuals and epidemiological investigations to describe the extent or prevalence of infection. SARS-CoV-2 immunoassays that detect IgM and IgG and are conducted in clinical diagnostic laboratories fulfill this function. This update outlines current information and recommendations uses and interpretation of SARS-CoV-2 testing modalities available in South Africa.

COVID-19 Diagnosis

Molecular tests that detect viral nucleic acids, COVID-19 or SARS-CoV-2 PCR tests remain the standard diagnostic test for symptomatic individuals.

These tests detect SARS-CoV-2 RNA in respiratory specimens of patients suspected of having COVID-19 disease. Viral RNA detection is done using a variety of gene probes or primers that recognise different genes of the SARS-CoV-2 RNA including structural genes (*N nucleopcapsid, E* envelope) and non-structural genes (*R* RNA-dependant-RNA-polymerase RdRp and the open reading frame 1 or ORF1) (See **Figure 1** outlining the viral components used in diagnostic tests). Detection of one or more gene confirms the presence of the virus and the sensitivities of the different tests using different genes done in different laboratories are equivalent.

Figure 1: Electron Micrograph SARS-CoV-2 Virus and Diagram Illustrating Virus Components used in Different Tests



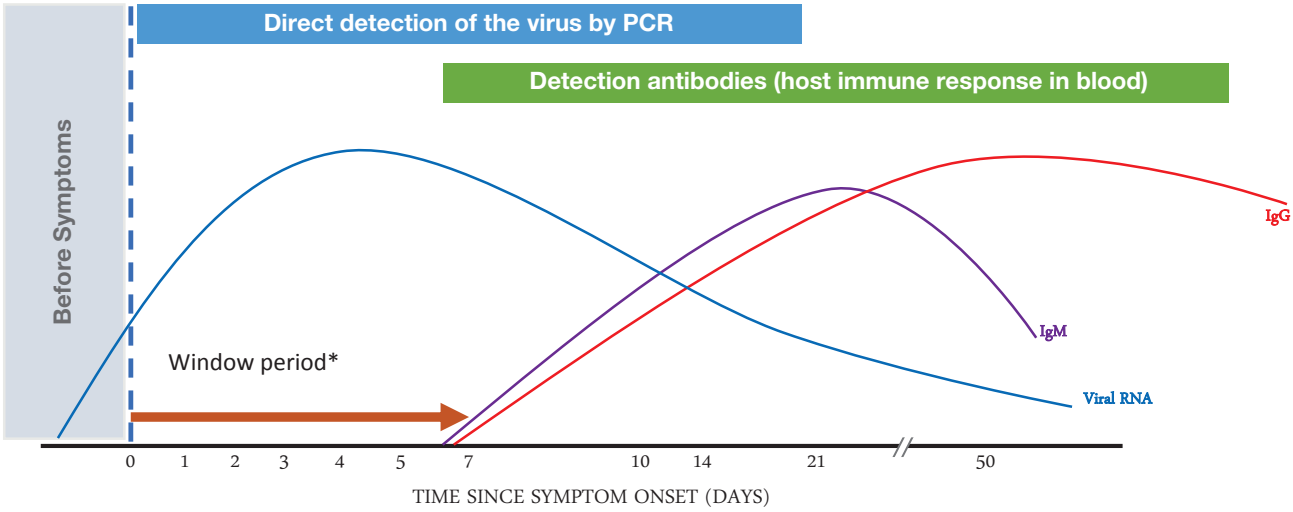
A negative PCR result indicates that SARS-CoV-2 RNA was not present in the specimen above the limit of detection of the assay. But a negative result does not exclude the possibility of COVID-19 and should not be used as the only indicator for patient management. False-negative results are possible and must be considered if the patient has a history of a recent exposure or the clinical presentation suggests that the diagnosis of COVID-19 is probable. Retesting of negative patients may be advisable in those symptomatic individuals with a high clinical suspicion of COVID-19. Due to the high specificity of SARS-CoV-2 PCR test false positives are unlikely and if a positive result is obtained it confirms detection of viral RNA and sequential or repeat testing is not recommended.

In general, based on information from many different experimental studies, SARS-CoV-2 viral RNA may;

- be detected 7 days before symptoms occur in individuals that become symptomatically infected
- continue to be detected for 25-30 days after symptom onset, but this detected virus is not infectious after 14 days
- be higher in more severely infected individuals
- asymptomatically infected individuals may have significant viral loads

See **Figure 2** for an estimated variation over time of diagnostic test markers)

Figure 2: Estimated Time Line for Detection of SARS-CoV-2 by Laboratory Tests



*window period = time from symptom onset to detection of antibodies

Both private and public-sector laboratories in South Africa have scaled up their capacity to meet the increasing demands for SARS-CoV-2 PCR testing. But this type of testing must be prioritized for patients that are symptomatic, health care workers, residents of long term care facilities or correctional facilities and shelters. Prioritization of patients is important because increased test demand may delay clinically important results, exceed the capacity of laboratories as well as the ability of manufacturers to supply test kits and reagents.

SARS-CoV-2 Serological Tests

There are different types of SARS-CoV-2 serology tests including, lateral flow tests (LFT's) or finger prick point of care (POC) tests, and enzyme linked immunosorbent (ELISA) or chemiluminescence (CLIA) immunoassays. These tests indirectly measure exposure or infection by detecting antibodies made in the normal immune response to a viral infection. The design of these serology tests is important, some tests only detect antibodies to nucleocapsid and some are designed to detect antibodies to the spike or S protein (See **Figure 1**). Serology tests are qualitative (ie positive or negative) and are unable to quantitate the levels of antibody detected nor are they able to indicate if the antibody is protective from re-infection.

The appearance of antibodies with viral infections usually follows the same pattern, IgM is produced first indicating a current or recent infection and then disappears. This immune response is followed by the production of IgG which lasts for many years and usually indicates immunity. With SARS-CoV-2 infection, this sequential appearance of antibodies is not normal or consistent and sometimes IgM appears around day 5 after infection, but in the majority of infected individuals both IgM and IgG occur together at around day 10-14 after infection ie the window period of this test is 10-14 days from symptom onset to detection of antibodies.

IgM antibodies disappear after about 5 weeks and IgG antibodies last for longer (see **Figure 2** for an estimated variation over time of diagnostic test markers). It is unknown at present, whether IgG antibodies that are detected by serological tests indicate immunity to re-infection or for how long protection to re-infection may last. However, detection of SARS-CoV-2 antibodies confirms exposure. Serological tests are designed to detect either IgG or IgM individually or combined (IgG+IgM) or other classes of antibodies like IgA.

Hundreds of different LFT devices are available in the market, some from reputable manufacturers and some from completely unknown entities. The performance of these LFT's, conducted in the field by non-laboratory qualified personnel and in their current design have *not been shown* to have sufficient sensitivity and specificity to be useful for either COVID-19 diagnosis or SARS-CoV-2 exposure. The rest of this document ONLY refers to high through put ELISA/CLIA tests done in accredited and certified laboratories with strict quality protocols in place.

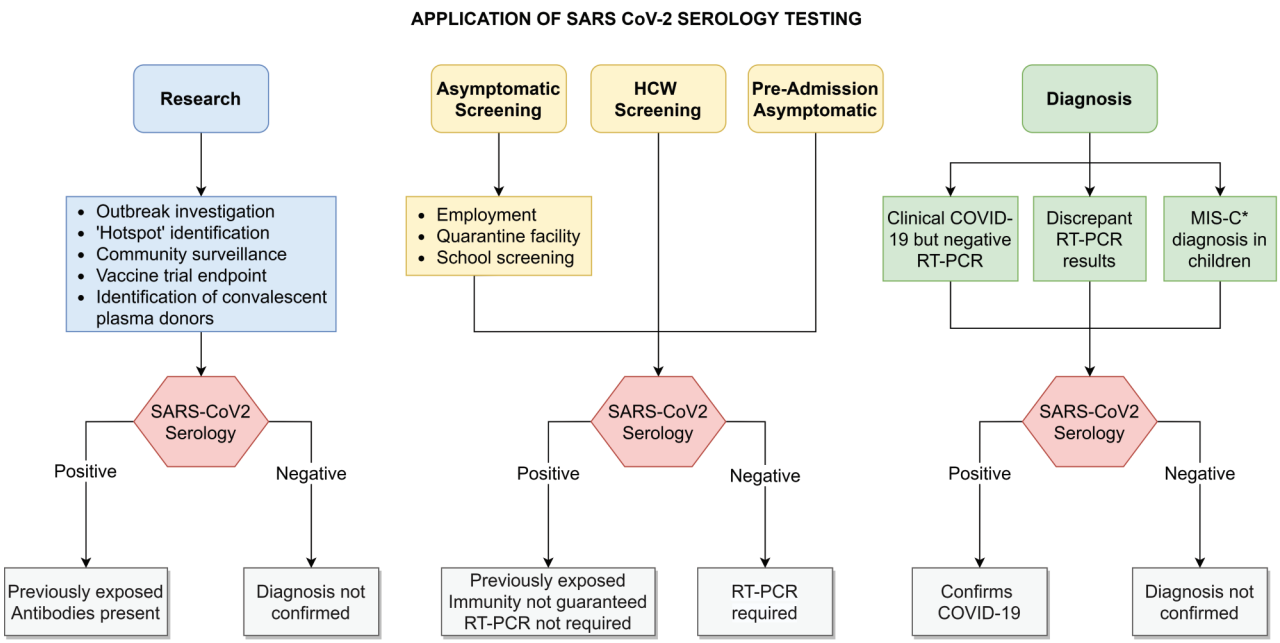
Figure 3 outlines the suggested algorithm and use of SARS-CoV-2 ELISA/CLIA antibody tests. It is important to note these tests are not recommended to diagnose infection except in certain circumstances when PCR tests will most likely be negative for example COVID-associated inflammatory syndrome or testing of CSF in patients with suspected SARS-CoV-2 encephalitis.

Detecting SARS-CoV-2 IgG antibodies does not infer immunity to the virus. Serology testing must be interpreted and directed to individuals that have a high "pre-test" probability to prevent false-positive results.

These tests may be used to;
- determine the rate of exposure or prevalence of infection in specific populations
- identify individuals who have been exposed to SARS-CoV-2 as possible donors of convalescent plasma as a possible treatment option for COVID-19 disease
- confirm infection in individuals that have a clinical illness that suggests COVID-19 but have negative PCR results
- assist with workplace and health care worker screening
- be used in SARS-CoV-2 vaccine research to determine vaccine efficacy

In low prevalence settings to reduce the likelihood of false-positive results, it is recommended maximising specificity by ensuring that testing is only used for individuals with a history of illness consistent with COVID-19 infection (ie a high pre-test probability), use those tests with high specificity, use sequential testing so that positive results are confirmed with a second antibody test with different antigen design combinations (nucleocapsid or spike protein, or receptor binding site RBS domain).

Figure 3 Applications of SARS-CoV-2 Serology



* COVID associated multisystem inflammatory syndrome

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