CORONAVIRUS DISEASE (COVID-19) OUTBREAK

Update on SARS-CoV-2 Laboratory Testing December 2020

As with all things COVID-19, the laboratory testing armamentarium available for SARS-CoV-2 continues to transform and develop, with improving test design and application plus greatly improved access. All together these contribute to better clinical management of cases and contacts, and ultimately, better prevention of new chains of transmission and outbreaks.

This update is a summary of the testing developments in the last few months. The main changes that have occurred are:

1. Introduction of SARS-CoV-2 antigen tests
2. Addition of SARS-CoV-2 IgM testing
3. Saliva samples for COVID-19 RT-PCR

SARS-CoV-2 Antigen Tests

Antigen testing offers distinct advantages for COVID-19 diagnosis, as it is quicker and cheaper than RT-PCR testing, and therefore more accessible. Antigen tests are immunoassays that detect the presence of specific viral antigens, which indicates current viral infection. Antigen tests are performed on nasopharyngeal swabs placed directly into the assay's extraction buffer or reagent. It is recommended that these tests are performed by accredited laboratories and trained laboratory personnel to ensure the best results. Antigen tests can be used for people of all ages, and return results after 20 – 60 minutes.

There is a growing body of literature to suggest that more frequent testing with cheaper and quicker tests like antigen tests, even if their sensitivity may be lower, is the best way to control the spread of SARS-CoV-2 [1, 2, 3]. Although testing capacity in South Africa has increased significantly, reagent supplies that can be sourced from international manufacturers may not always meet the country's requirements. Clinically relevant turn-around times may therefore be extended from time to time. This is especially an issue with surge activity, which further constrains PCR testing resources. It is during these geographically- and epidemiologically-linked outbreaks where turn-around times are critical for contact tracing and testing, that SARS-CoV-2 antigen tests may be applied. Sensitivities of the antigen tests being offered at Lancet Laboratories are between 75 – 80% and specificities are high at 99.9%. False-positives are therefore rare.

However, because antigen tests for SARS-CoV-2 may be less sensitive than PCR tests that detect nucleic acid, their technical performance and interpretation are critical. International guidelines stipulate that COVID-19 antigen tests must be conducted by accredited laboratories and trained laboratory professionals; furthermore, these tests are designed to be used primarily in **symptomatic individuals**. There are limited data to guide the use of rapid antigen tests as screening tests in asymptomatic persons to detect or exclude COVID-19, or to determine whether a previously confirmed case is still infectious. The current WHO and CDC recommendations advise antigen testing with caution in asymptomatic individuals [4].

Four scenarios have been defined in the South African consensus document on antigen testing for the use of rapid antigen tests [5]:

a. **Symptomatic individuals** – in a confirmed outbreak situation, antigen tests can be used for contact tracing, to triage patients in emergency rooms, or test in-patients in ICU's or wards who become ill with symptoms suggestive of COVID-19.

b. **High-risk groups, healthcare worker/essential worker screening in high prevalence settings** – antigen tests can be done regularly because the test is affordable, and frequent testing may identify infected individuals earlier and prevent super-spreader events.
c. **Contact tracing** – in a low prevalence setting, antigen testing of all contacts of confirmed cases (symptomatic and asymptomatic) can assist to implement quarantine and isolation protocols, and rapidly terminate chains of transmission.
d. **Port of entry screening** – this is currently a WHO conditional recommendation; however, the South Africa Port Health Authority has implemented this strategy to screen individuals from low prevalence areas who either do not have PCR results on arrival, or are symptomatic [6]. This test has the advantage of quick turn-around times, permitting rapid institution of quarantine and isolation protocols.

SARS-CoV-2 Antibody Tests

Lancet Laboratories introduced SARS-CoV-2 antibody testing in August 2020, following SAHPRA approval of a limited number of high throughput SARS-CoV-2 IgG test kits. These detect IgG directed against the nucleocapsid (N protein). Of the > 11 000 tests done to date in our laboratory, 24.6% of samples have tested positive for IgG antibodies. A study performed in Cape Town on antenatal and HIV-1 clinic surveillance samples detected a higher seroprevalence [7].

We await regulatory approval of immunoassays that detected antibodies directed against the viral spike protein (S protein). S protein antibodies may be neutralising, and are likely to provide protection against re-infection. The currently approved COVID-19 vaccines are designed to generate antibodies to the S protein; thus, measuring anti-S protein antibodies post-vaccination may become a clinically useful indicator of immunity.

Tests for IgM antibodies have recently become available. Testing for IgM is currently a requirement for travel to China, where entry regulations require recent negative RT-PCR and IgM test results. The IgM antibody test on its own is not regarded as sufficient diagnostic evidence of recent infection. However, it may be of clinical relevance when used in conjunction with repeat RT-PCR and SARS-CoV-2 IgG tests in patients where there is clinical suspicion of COVID-19 but negative initial PCR results [8].

RT-PCR using Saliva Samples

There has been significant debate about different specimen types suitable for COVID-19 PCR testing. Some studies have suggested that the sensitivity of PCR tests is higher in lower respiratory tract samples of symptomatic patients as these have higher viral loads [8]. Whereas other studies, in which paired sampling, i.e. nasopharyngeal and oropharyngeal specimens, tested simultaneously have shown that viral RNA levels are higher and more frequently detected in nasal (66%) compared with oropharyngeal (35%) specimens [9,10].

Self-collected saliva specimens are ideal in many situations, decreasing the risk of exposure of healthcare workers, and increasing the speed in which samples can be collected and sent to the laboratory. Studies have shown that the accuracy of PCR is good (94%) with certain self-collected specimens (i.e. nasal swabs and saliva specimens), as is the sensitivity (85%), when compared to healthcare worker-collected nasopharyngeal swabs [10]. The optimal method for saliva collection is currently unknown, and instructions on collection need to be communicated clearly to the patient.

Lancet Laboratories is investigating the utility of saliva samples for SARS-CoV-2 RT-PCR testing.

Table 1 summarises the recommended uses of all existing COVID-19 (SARS-CoV-2) laboratory tests currently available for quick reference.

Table 1. Diagnostic Tests for COVID-19/SARS-CoV-2 Infection

Test Type	Clinical Uses	Specimen type	Performance	Comments
RT-PCR	Diagnosis of current infection	Respiratory tract samples (NP / OP / sputum / mid-turbinate nasal)	* Sensitivity and specificity high. * Performance depends on specimen quality and timing in course of infection. * False-negative rate 5 – 30%. * False-positivity rate low, but can detect non-infectious viral genetic material up to 30 days	* TAT depends on test demand. * TAT 6 – 10 hours priority urgent, 24 – 48 hours pre-admission, travel. * TAT of 4 days or more not useful to guide appropriate clinical and public health interventions.
Serology (Ab) IgG/IgM	Diagnosis of prior infection (or vaccination)	Blood	* Laboratory-based immunoassays have superior sensitivity to point-of-care tests. * Sensitivity of IgM at 5 – 7 days after infection 70 – 85%. * Sensitivity IgG at 10 – 14 days after infection 85 – 90%. * Specificity of both IgM and IgG is high (99%). * No current evidence of cross-reactivity with other human coronaviruses, this remains unconfirmed and theoretical.	* Viral targets variable depending on kit design (N/S protein or both). * Should be performed by accredited laboratories and experienced staff. * Done as IgG only, IgM only, or combined IgG+IgM depending on manufacturer. * Uncertain if a positive antibody IgG test indicates immunity against further infection or protective vaccine response for COVID-19 vaccine recipients.
Antigen (Ag)	Diagnosis of current infection	Dry nasopharyngeal swab (no viral transport medium)	* Immunoassay generally less sensitive compared to RT-PCR, depending on which assays are compared. * Must be performed by laboratory personnel.	* TAT approx. 1 hour. * Cheaper than RT-PCR. * Increased access and faster TAT compared to PCR. * Should be performed by accredited laboratories and experienced staff. * Data on asymptomatically infected individuals is limited.
Combination testing RT-PCR IgM IgG SARS-CoV-2 Ag	Diagnosis of current infection or previous exposure (depends on which tests are included)	Respiratory tract samples (NP / OP / sputum / mid-turbinate nasal) And Blood	* A single positive PCR or Ag test confirms infection.	* If initial testing is negative, and clinical suspicion remains high, a second test of the same type or a different type of test enhances the diagnostic yield.

NP = nasophyngeal; OP = orophyngeal; TAT = turn-aroundtime; Ab = antibody; N = nucleocapsid protein; S = spike protein; Ag = antigen.

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