THE USE OF ANTIGEN TESTING FOR THE DIAGNOSIS OF SARS-COV-2 IN SOUTH AFRICA

Foreword

Efficient and timely testing for Covid-19 is not only required for individual patient management, but at a population level is a key component of public health efforts to control the pandemic. The World Health Organization (WHO) has called on all countries to ramp up testing programmes. Dr. Tedros, Director-General of WHO, made it clear at a media briefing in Geneva on 16 March 2020 "We have a simple message for all countries: test, test, test. Test every suspected case."

Whilst PCR testing remains the gold standard for diagnosing SARS-CoV-2 infection, antigen testing provides an important alternative. This document explores the impact of antigen testing for various use cases and guides the implementation of these tests across different settings. The document also incorporates guidance regarding eligibility criteria for testing, and testing are ports of entry.

It is hoped that the guidelines will contribute to increased testing, which will assist us to identify and respond to outbreaks in a timely and effective manner.

I would like to thank all those who contributed to the development of this guideline.

DR SSS BUTHELEZI
DIRECTOR-GENERAL: HEALTH
DATE: 11 DECEMBER 2020

Introduction

The most accurate method to diagnose infection with SARS-CoV-2 is molecular nucleic acid amplification technology (NAAT), which detects the presence of SARS-CoV-2 genetic material in respiratory specimens. These real-time PCR tests are sensitive (>95%)[1] but are often limited in supply, require a laboratory testing environment and turnaround time to result is often >24hrs, reducing their clinical relevance and utility for epidemic control. Antigen tests detect structural proteins of the SARS-CoV-2 virus, with the majority tests based on lateral flow technology that either requires a visual read or the use of a reader for interpretation of a control and test line. These rapid tests are performed in < 30mins at point of care, thereby enabling immediate patient care decisions, meet testing demands in resource limited settings and increasing access to testing by supporting service decentralization.

PCR has a broad window of detecting SARS-CoV-2 infection (Figure 1), but less sensitive antigen tests can be used on pre-symptomatic (1-3 days before symptom onset) and early symptomatic phases of illness when individuals are likely to be most infectious[2]. As per WHO recommended minimum performance requirements, antigen tests with >97% specificity and >80% sensitivity may be used for diagnosing infection with SARS-CoV-2, where no nucleic acid amplification tests are available or have prolonged turnaround times.
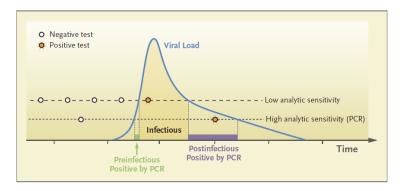

Figure 1: Testing sensitivity profile based on viral concentrations during infection with SARS-CoV-2. High-frequency testing with low analytical sensitivity versus low-frequency testing with high analytical sensitivity[3]

A targeted testing strategy was introduced in July 2020 to accommodate the country's constrained testing capacity, to deal with the testing backlog and to ensure that those categories of patients with an urgent clinical need were prioritised for testing. Symptomatic hospitalitsed patients as well as health care workers were therefore prioritised for testing

However, this guidance was updated in October 2020 in order for South Africa to utilise its expanded Covid-19 testing resources to support all components of the Covid-19 response. The updated testing criteria are shown in Box 1. People entering South Africa who do not have proof

---

[1] WHO TPP v0.1
[2] Antigen detection in the diagnosis of SARS CoV-2 infection using rapid immunoassays. WHO Interim guidance. 11 September 2020.
[3] (Mina, M.J. et al. Rethinking Covid-19 Test Sensitivity — A Strategy for Containment. NEJM. DOI: 10.1056/NEJMp202563

of a negative PCR test taken less than 72 hours before departure have also been identified as an important category of people who will require access to testing.

The following categories of people should be tested in both the public and private health sectors:

1. **Hospitalised patients**
   a. **Symptomatic[4] patients must be prioritised, and test results should be received within 24 hours**
   b. All other patients should be tested on admission.
2. **Any person with symptoms where Covid-19 infection is considered to be a possible cause**.
   a. Persons at high risk for infection or poor outcomes, e.g. health care workers, those aged > 60 years, those with co-morbidities, pregnant women, should be prioritised.
   b. Other symptomatic[4] persons presenting to health facilities, as well as those identified as being symptomatic during health facility, workplace, school or other screening, should be tested.
3. **Individuals who are close contacts of confirmed cases, including asymptomatic[5] contacts.** Whilst a close contact is generally defined as contact within one metre of a Covid-19 confirmed case for >15 minutes without PPE (no face cover/eye cover), alternative definitions should be used in some high-risk settings (e.g. clusters/outbreaks). Please refer to the latest "National Guidelines on Contact Tracing for Covid-19" and "Guidelines for symptom monitoring and management of essential workers for COVID-19 related infection" for further details.
4. Post-mortem testing should be conducted in line with current guidance.

The following individuals or groups should not be tested (unless they fall into one of the priority groups):

1. Asymptomatic people including employees/learners for purposes of returning to work/school
2. Selected groups e.g. sports persons
3. Patients meeting de-isolation criteria as per National Department of Health clinical guidelines should not be re-tested.

Box 1: Revised testing criteria as contained in the Updated Prioritised Covid-19 testing guidance issued on 20 October 2020.

Detection of viral RNA using a SARS-CoV-2 rRT-PCR assay is the recommended method for the diagnosis of an active SARS-CoV-2 (Covid-19) infection in South Africa. However, the turnaround time for central laboratory-based SARS-CoV-2 rRT-PCR results is often greater than 48 hours, limiting the ability to effectively isolate, treat, and contact trace in a timely fashion.

---

[4] "*Symptomatic*" for the purposes of this list means a person with symptoms suggestive of Covid-19 following the current definition of a person under investigation (PUI) for Covid-19.
[5] "*Asymptomatic*" for the purposes of this list means a person without symptoms suggested by the current PUI definition- although this person may have other symptoms

Detection of viral proteins through antigen-based point-of care (POC) tests[6] is therefore an alternative option for diagnosing active infection that may allow for faster, easier, less expensive, and more widespread testing.

This document explores the impact of antigen testing for various use cases and guides the implementation of these tests across different settings.

Potential use cases for antigen tests

In absence of antivirals and a prophylactic vaccine for Covid-19, the primary goal of diagnostics is to inform and reduce spread of transmission. Given the infectiousness of SARS-CoV-2, starting in the 1-5 days prior to those that develop symptoms and throughout for those who have mild symptoms or are asymptomatic, there is a need for fast turnaround of results (same day) to institute isolation of cases as well as tracing of contacts. Thus rapid antigen tests are potentially useful as they can inform healthcare workers and individuals their infection status at point-of-care for individual-level action (triage/isolation) and these tests can be used to inform community level response (surveillance). Furthermore, rapid antigen tests allow for decentralization of SARS-CoV-2 testing thus increasing testing coverage which can provide policy makers higher confidence to institute adaptive policy response at local/regional level. Although rapid antigen tests allow for point of care testing, it is important to note that they are performed on respiratory specimens and so would still need to be collected by appropriately trained staff under appropriate biosafety precautions.

We assume four primary use cases for antigen tests to inform and reduce SARS-CoV-2 transmission:

- *Symptomatic patients (strong recommendation by WHO)*
In areas where community spread has been detected, we assume programs will test all suspected cases with severe symptoms as well as a proportion of mild/moderate cases that qualify based on other risk factors. This includes screening of at-risk individuals in the community and within closed or semi-closed groups (e.g. prisons, nursing homes, schools, workplaces, dormitories, etc), We expect this use case to be a high prevalence setting in confirmed outbreak settings and thus results should be interpreted accordingly (Figure 2). The point of care test in this scenario would allow healthcare workers to quickly triage patients and manage them accordingly.
In areas where either community spread has not been detected or there is an unconfirmed outbreak, we assume programs will test all symptomatic cases presenting at hospitals and health facilities with the objective of rapidly detecting new case clusters. We expect this use case to be a low prevalence setting and results should be interpreted accordingly (Figure 2). The point of care tests in this scenario would allow policy makers to adjust lockdown restrictions at local/regional level.

---

[6] These immunoassays utilize antibodies against the nucleoproteins of SARS-CoV-2 to detect viral antigens.

- *High Risk groups (strong recommendation by WHO)*

In areas where community spread has been detected, <u>healthcare workers and essential workers</u> would need to be screened regularly as they face an increased risk of contracting SARS-CoV-2. As these populations can act as super-spreaders, there is a need to identify them earlier so they can be isolated/quarantined. Due to <u>increased risk in these populations, we expect this use case to be a high prevalence setting</u> especially in symptomatic healthcare workers and results should be interpreted accordingly (Figure 2).

- *Contact tracing (strong recommendation by WHO)*

Depending on human resource availability, we assume programs will test all contacts of the cases (<u>symptomatic and asymptomatic</u>) and implement quarantine and isolation protocol to terminate transmission chains. We expect this use case to be a low prevalence setting and results should be interpreted accordingly (Figure 2). Antigen tests with high specificity and sensitivity should be prioritized for this use case.

- *Port of entry screening (conditional recommendation by WHO)*

We assume aggressive screening of travelers crossing borders to ensure new cases are not seeded by the imported cases. Due to <u>high volume of travelers at the port of entries, we expect this use case to be a low prevalence setting</u> and results should be interpreted accordingly as low specificity tests may yield a high false positive rate. Nucleic-acid based POC tests have higher specificity and may offer performance advantages, but may be too slow and costly to allow prompt action and the scale needed. This use case for antigen tests has however been included in national policy. Currently WHO does not strongly recommend this use case <u>unless there is data available from high-quality studies that confirms >99% specificity for the antigen test used in this setting</u>. High sensitivity is also important, ideally >85-90% to reduce the risk of missing infected individuals. Antigen tests for this use case should be selected based on these performance thresholds as far as possible.

- *Routine screening in schools and workplaces (conditional recommendation by WHO)*

Screening for individuals entering schools and workplaces may be a critical strategy to reopening the economy and staying open. Due to high volume of tests required in these settings, particularly for asymptomatic clients, we expect <u>this use case to be a low prevalence setting</u> and results should be interpreted accordingly. Currently WHO does not recommend this use case unless data from high-quality studies confirms >99% specificity in the antigen tests used. Implementing this use case using nucleic-acid based tests is cost prohibitive and maybe plausible through more affordable solutions such as antigen-based tests. <u>The preadmission screening of urgent surgical or medical cases in hospitals may be included in this category.</u>

Other practical considerations for Ag RDT implementation are:

(i)   Pilot sites should be selected where NAAT is available, such as NHLS and clinical partner HCW screening (one specimen collected for RDT and paired specimen for confirmatory and technology monitoring) and hospital admissions.

(ii)   If NAAT confirmation is not available, results should be interpreted in conjunction with clinical symptoms and disease prevalence. These settings may also include mobile vehicles and community point of care.

Types of SARS-CoV-2 antigen tests

There are multiple antigen POC tests available for SARS-CoV-2:
- Simple, manually run rapid lateral flow tests without any device or instrument (also known as visual rapid diagnostic tests (RDTs)) – e.g. Abbot Panbio and SD Biosensor Standard Q
- Simple and manually-ran RDTs read with a reader – e.g BD Veritor, Quidel Sofia, SD Biosensor Standard F
- Small, simple POC instrument rapid lateral flow tests that are both run and read by the device- e.g. LumiraDx.

Preliminary studies indicate that POC device-based assays have higher sensitivity compared to visual RDTs. However, as more sensitive antibodies against SARV-CoV-2 antigens become available, visual RDTs may achieve parity with device-based assays. The specificity of visual RDTs approved on the WHO Emergency Use List is high and may have comparable performance with device-based assays.

A number of test kits from Abbot, SD Biosensor and Lumira will be donated to South Africa for validation and catalytic procurement. A summary of these technologies are highlighted in table 1 below.

Table 1: Technology Overview

Supplier	TAT (minutes)	Swab Collection Type	Detection method
Abbot Panbio	15	Nasopharyngeal swab	Chromatographic immunoassay
SD Biosensor (Standard Q)	15	Nasopharyngeal swab	Chromatographic immunoassay
LumiraDx	12	Nasal swab	Microfluidic immunofluorescence
SD Biosensor (Standard F)	30	Nasopharyngeal swab	Fluorescent immunoassay

Sensitivity and specificity

Current draft guidance[7] from the World Health Organization indicates the minimum performance of an antigen test to be ≥80% sensitivity and ≥97% specificity. While it is always preferred to have the highest possible performance levels, it is important in a public health context to assess the benefits and risks of lower performance tests in relation to patient outcomes. High-sensitivity tests reduce false negatives, particularly in higher prevalence settings, while higher specificity tests are most critical to reduce numbers of false positives in low prevalence settings. It is important to note that the decline of antigen levels in samples taken beyond 5 – 7 days post onset of symptoms is expected to yield false negatives with some antigen tests, which is a significant limitation of antigen testing. Some antigen tests claim to have high sensitivity for a longer detection period of up to 10-12 days post symptom start, e.g. LumiraDx.

A recent systematic review showed that sensitivity of these Ag RDTs varied considerably across studies (from 0% to 94%) with an average sensitivity of 56.2% (95% CI 29.5 to 79.8%) while specificity was high with an average specificity of 99.5% (95% CI 98.1% to 99.9%; based on 8 evaluations in 5 studies on 943 samples)[8]. The sensitivity of the antigen tests is higher when viral loads are higher (low cycle threshold (Ct) in RT-PCR), which is usually within the first 5 days following symptom onset, therefore timing of testing is crucial.

Limitations and Exceptions

- For the visual RDTs (e.g., Abbott Panbio and SD Biosensor) an urgent solution is needed for connectivity and data collection to ensure information management. Abbott does provide a connectivity app for its PanBio test.
- Patient history and management needs to be taken into account
- There is currently insufficient data to confidently recommend where these tests should be used and inform the testing criteria and guidelines.

Evaluation Framework

Table 2: Risk-benefit evaluation framework for moderate sensitivity tests

Testing option	Potential benefits	Potential risks / downsides
SARS-CoV-2 RT PCR test	• **High accuracy** testing option and the test of choice for clinical diagnosis.	• **Long turnaround times** limit the ability to quickly isolate, treat, and contact trace • Likely to face **supply, capacity, and/or cost constraints** that limit testing access

---

[7] World Health Organization, Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays, Interim guidance, 11 September 2020

[8] References: Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, Emperador D, Takwoingi Y, Cunningham J, Beese S, Dretzke J. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database of Systematic Reviews. 2020(8).

		• *Limited ability to drive local/regional level policy decisions* due to limited testing coverage
Antigen RDT or POC device	• **High accuracy** testing option for high sensitivity tests • **Faster turnaround** times • **Lower cost** than PCR • **Greater access to testing,** particularly in areas further away from PCR labs • **Can drive local/regional level policy decisions** through decentralization of testing to lower level health facilities • **Faster identification of cases and contacts** for quarantine/isolation **to control the epidemic**	• For moderate sensitivity tests, a **small number of false negative results can occur**, especially where there is community spread and in high prevalence settings such as inpatient wards and symptomatic HCW • **Additional training** and data management needed to decentralize testing to lower level health facilities and cadres • Use of **high specificity** tests will reduce the occurrence of false positive results especially in low prevalence settings

Interpretation of antigen results

Proper interpretation of antigen results is important for both clinical management of patients and for assessing the SARS-CoV-2 epidemic. As highlighted above the accuracy of results depends largely on the conditions under which the results are interpreted. Understanding these conditions can help minimize being misled by false positive or false negative results.

- *High prevalence settings*

The positive predictive value of an antigen test is the highest when SARS-CoV-2 prevalence is high in the population being tested. In this population a positive test can be interpreted as likely SARS-CoV-2 infection and appropriate treatment initiation and prevention and control measures should be followed. The negative predictive value of an antigen test is lowest when prevalence is high and false negative results are more likely to occur. A negative could imply that the infection is unlikely if there are no clinical signs or epidemiological connection to SARS-CoV-2. If there are clinical and or epidemiological indications for Covid-19 then additional testing could be considered, particularly in settings with high risk for downstream transmission. While considering need for additional testing, time of onset of symptom should also be considered as viral load in upper respiratory tract peaks within the first week of infection, followed by a gradual decline over time. In these patients, additional testing could be done on lower respiratory sample using RT-PCR or an antibody test can be considered for patients presenting >10 days post onset of symptoms.

- *Low prevalence settings*

The positive predictive value of an antigen test is the lowest when SARS-CoV-2 prevalence is low in the population being tested. In this population, false positive results are more likely

to occur and thus a positive test would need to be interpreted with clinical and epidemiological link to an outbreak or travel to areas with Covid-19 spread. However, if confirmation of infection is desired then additional testing on RT-PCR or another high sensitivity/high specificity test would be needed. Meanwhile a negative results implies that the infection is unlikely if there are no clinical signs or epidemiological connection to SARS-CoV-2 (Figure 2). To minimize the false positives, only assays with high specificity should be used, ideally assays with ≥99% specificity.
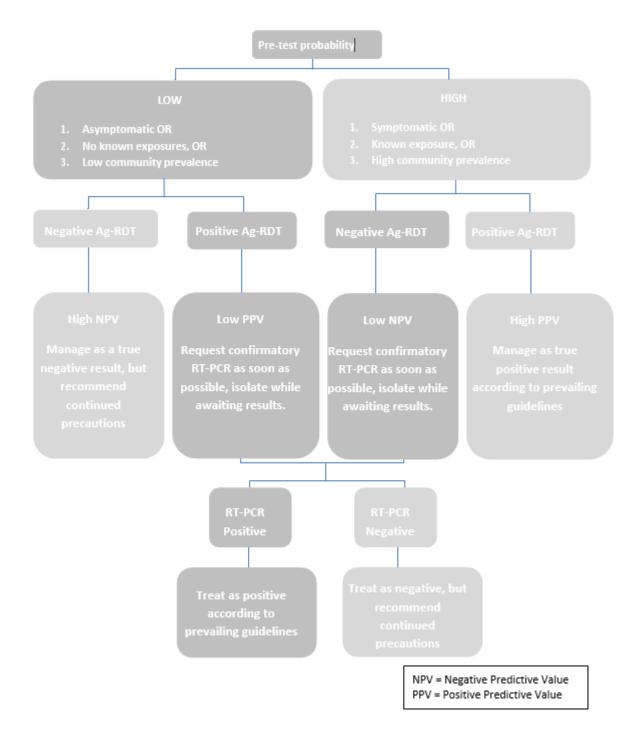

Figure 2: Proposed testing algorithms based on prevalence settings

Antigen testing implementation plan

Currently, the demand for the SARS-CoV-2 RT-PCR testing capacity is acceptable, however there are circumstances where rapid antigen testing would be desirable, especially given the anticipated risk of resurgence. Point of care antigen testing could improve health outcomes, improve contact tracing efforts and assist in controlling the spread of the virus, when implemented correctly.

Due to a lack of sufficient in-country validation data, as well as sub-optimal access to test kits for validation and low sample sizes, a cautious, phased approach is recommended.
Following validation and in-field studies, further guidance on implementation will be provided.

Phase 1: Ports of entry

As per the press release, incoming travelers who don't have a SARS-CoV-2 RT PCR test result on arrival will be screened, and

1. Symptomatic travelers will be refused entry OR be required to complete a 10 day isolation in South Africa at a quarantine facility at their own cost and will receive a SARS-CoV-2 RT-PCR test, as they are deemed high risk.
2. All <u>asymptomatic travelers will receive an antigen rapid diagnostic test</u>.
3. It is proposed that the first 500 travelers (distributed per port of entry), both symptomatic and asymptomatic, be tested using both the Ag RDT and RT-PCR for verification purposes and these data be pooled to contribute to local validation data.

Travelers on the exemption list like truck drivers and day scholars/students, will receive an antigen test. The frequency of testing in these asymptomatic populations still needs to be established. It is proposed that frequent use of cheaper, simple and rapid Ag detection tests has a potential to limit transmission by early detection of infection, even if their analytic sensitivities are vastly inferior to those of benchmark tests. Simulation studies show that strategies with less frequent testing like once-a-week, may be sufficient in settings with low community incidence, especially when implemented with non-pharmaceutical interventions like social distancing, masking, regular hand washing or use of alcohol based sanitisers.[9]

Ideally for low prevalence populations, positive Ag tests need confirmation using RT-PCR. However, the cost of a PCR tests in most settings is a likely to be a prohibiting factor.

---

[9] Bradley EH, An MW, Fox E. Reopening Colleges During the Coronavirus Disease 2019 (COVID-19) Pandemic—One Size Does Not Fit All. JAMA Network Open. 2020 Jul 1;3(7):e2017838-.

> **Management of Ag RDT results:**
>
> **NB: Notification of positive cases to NMC is mandatory.**
>
> **NB: All results (positive and negative) must be submitted to NHLS and NICD (private sector) as per current RT-PCR results submission.**
>
> **NB: Clear process of linking positive Ag RDT results to the contact tracing team must be established.**

Implementation of Ag RDT testing will require engagement between NDoH (and other government bodies), NHLS and Private Sector laboratories to ensure Ag RDTs can assist in guiding patient management, public health decision making and surveillance of Covid-19. The implementation model may be guided by the national POC testing policy[10]. This POC-T policy (although not designed for Covid19) outlines models for national coverage (address gaps in current services), total decentralization (large-scale) and niched settings (specific environments for target populations).

---

[10] Implementation of Point of Care Policy. A national policy document to guide the provision of quality POCT services in South Africa, 2018/12/19