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SPECIAL INTEREST GROUP OF THE SOUTH AFRICAN MEDICAL ASSOCIATION

SARS-CoV-2 Antigen Test Protocol and Guidance

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The current recommended method for the detection of SARS-CoV-2, the virus that causes COVID-19, is the reverse transcriptase polymerase chain reaction (RT-PCR) test, which is a type of nucleic acid amplification test (NAAT). While this method is more sensitive than other test modalities for the detection of SARS-CoV-2, even RT-PCR tests may produce false negative results in infected individuals. Due to limitations related to complexity of performing these tests, global shortages in test kits and supplies, the requirement for specialised laboratory equipment, high test cost and the potential for delayed turnaround times, there has been a drive towards the development of rapid point of care test methodologies for the detection of SARS-CoV-2. Immunoassay devices that detect SARS-CoV-2 antigen have been earmarked as a possible solution for rapid near-patient testing. Following their release to market, however, SARS-CoV-2 antigen assays have shown variable performance during evaluations. With sensitivities ranging from 11 to 94%, SARS-CoV-2 rapid antigen assays are less sensitive than NAAT tests, with best sensitivity demonstrated at higher viral loads. Test specificity has at least been consistently high despite the wide variation in sensitivity. The WHO recommends a sensitivity of >80% and a specificity of >97% as minimum performance requirements for SARS-CoV-2 antigen tests.

While some of the SARS-CoV-2 antigen assays show promise as rapid and more cost effective alternatives to NAAT assays in the diagnosis of COVID-19, the NPG would like to highlight the following issues which should be taken into consideration prior to implementation of these assays in practice:

- SARS-CoV-2 antigen tests are performed on respiratory specimens, particularly nasopharyngeal swabs, and therefore poor sample quality may produce false negative results. For this reason sample collection would require staff trained in the methodology.
- Performing these tests involves processing of potentially infectious specimens and therefore presents biosafety risks. As such, personal protective equipment, biohazard waste bags and good ventilation are essential components for performing these tests.
- Although these tests are easier to perform than NAAT tests, the requirements as per manufacturer's instruction still need to be followed precisely, documentation of results need to be done carefully, and factors such as storage and shelf-life need to be managed actively.

- It is of critical importance that test kits undergo verification at the site of testing to ensure that the tests perform as well as indicated by the manufacturer. This is done by all ISO certified accredited laboratories before tests are implemented. Ongoing quality assurance checks should also be implemented as per standard laboratory practice for point of care tests, to ensure that there are no lot or device problems.
- The sensitivity of the antigen tests is higher when viral loads are higher (low cycle threshold (Ct) in RT-PCR), which is usually one to two days prior to, and 5 days after, symptom onset. Timing of testing is therefore crucial.
- Manual interpretation of faint lines may be subjective and particularly difficult for inexperienced operators.
- Low pre-test probability/low population prevalence increases the likelihood of false positive results being recorded., This will limit the use of these tests for screening purposes, such as at points of entry, blood donation or elective surgery admissions in hospital settings.
- There is still a lack of data regarding performance of these tests in asymptomatic individuals.

The WHO has released an Interim Guidance document for Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays on 11 September 2020, which makes recommendations for the appropriate use of these tests. The NPG wishes to highlight the following recommendations from this document in particular:

- Clinical use should only be considered in settings where COVID NAAT is unavailable or where prolonged turnaround times preclude clinical utility of NAAT.
- Where possible, all samples that test positive on rapid antigen tests (or at least a subset of these) should be transported to laboratories with NAAT capability for confirmatory testing.
- A negative antigen result cannot completely exclude an active COVID-19 infection, and therefore repeat testing, or preferably confirmatory testing, using NAAT should be performed whenever possible, particularly in symptomatic patients.

On 11 December 2020, the South African Department of Health released a guidance document detailing the use of antigen testing for the diagnosis of SARS-CoV-2 in South Africa. This document reiterates that SARS-CoV-2 RT-PCR assay is the recommended method for the diagnosis of an active COVID-19 infection, and that the ability to effectively isolate, treat, and contact trace in a timely fashion will mostly be limited where the turnaround time for RT-PCR

results is **greater than 48 hours**. This document lists five primary use cases for antigen tests within the South African setting:

1. Screening of symptomatic individuals in the community and within closed or semi-closed groups (e.g. prisons, nursing homes, schools, workplaces, dormitories, etc.) *in confirmed outbreak settings.* These would be high prevalence settings and point of care test in this scenario would allow healthcare workers to quickly triage patients and manage them accordingly.
2. Regular screening of high risk groups e.g. health care workers and essential workers, especially if symptomatic.
3. Contact tracing, to test all contacts of the cases (symptomatic and asymptomatic) and implement quarantine and isolation protocol to terminate transmission chains.
4. Port of entry screening, which is not strongly recommend by the WHO unless there is data available from high-quality studies that confirms >99% specificity for the antigen test used in this setting.
5. Routine screening in schools and workplaces, but due to low prevalence setting the test specificity needs to be high (>99%).

Importantly, this document states that there is currently insufficient data to confidently recommend where these tests should be used and inform the testing criteria and guidelines. As such these tests are not currently recommended for routine diagnostic use outside the aforementioned use cases.

The pre-test probability is a crucial factor to be considered when interpreting the SARS-CoV-2 antigen tests, as this will affect the positive and negative predictive values of these tests. The recommendations for confirmatory testing by NAAT will vary depending on this as follows:

- **High prevalence settings and/or high pre-test probability**, e.g. symptomatic person in a setting with established community transmission: The positive predictive value (PPV) is high, so a positive result can be managed as a true positive, while a negative result should prompt confirmatory testing by RT-PCR.
- **Low prevalence settings and/or low pre-test probability**, e.g. asymptomatic travellers with no known exposure: In this instance the negative predictive value (NPV) would be higher, as such negative results need no confirmation, but the risk of false positive results exists despite good specificity. Confirmation of positive results by RT-PCR is recommended.

It is important to note that notification of positive cases to NMC is mandatory. All results (positive and negative) must be submitted to the NICD as per current RT-PCR results submission.

NPG SARS-CoV-2 Antigen Test Protocol

The NPG recommends the following protocol to be followed in the case of screening for COVID-19 by means of SARS-CoV-2 antigen tests:

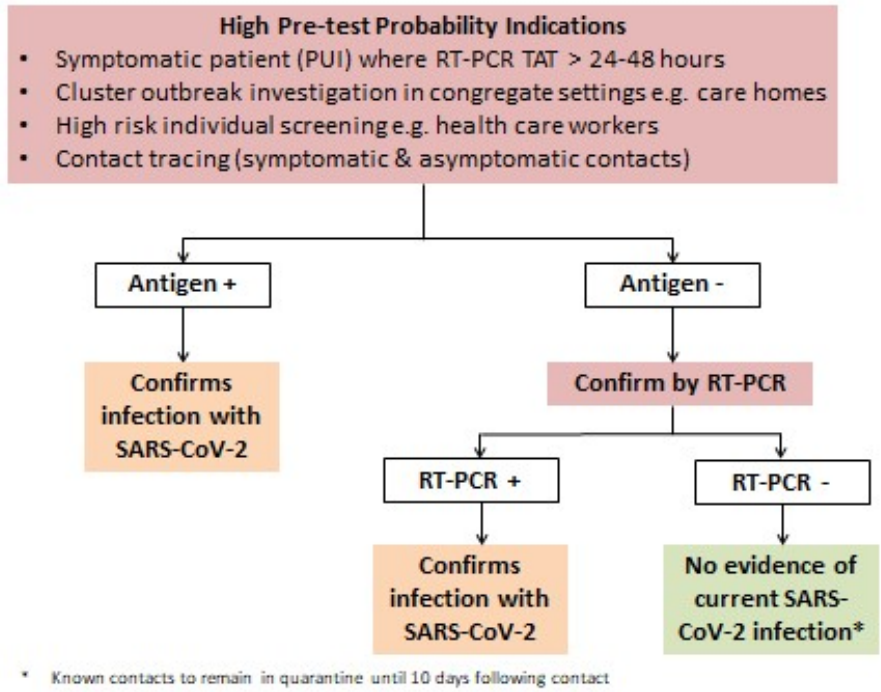

Figure 1: Testing protocol by means of SARS-CoV-2 Antigen tests for high pre-test probability indications

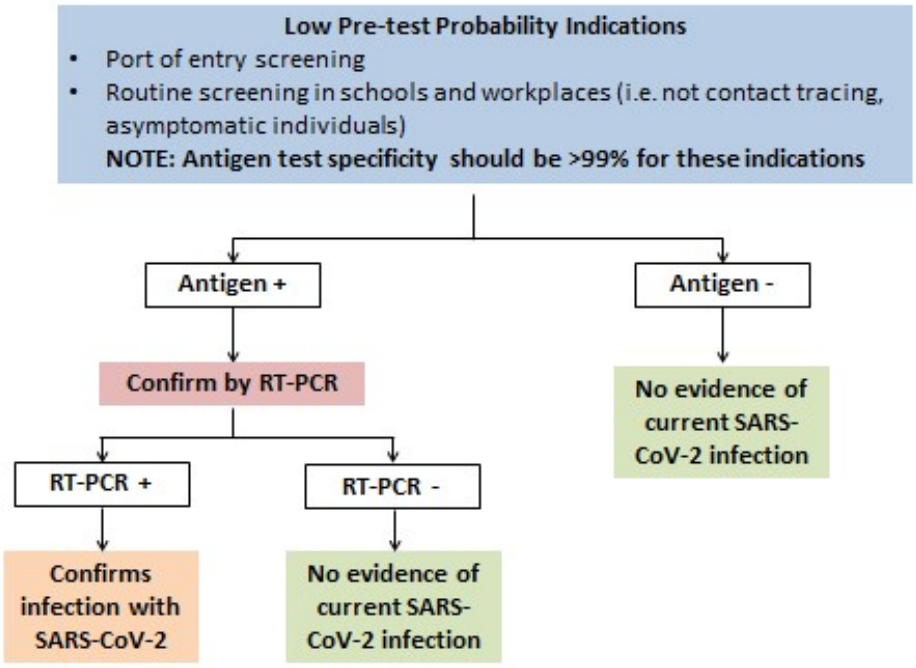

Figure 2: Testing protocol by means of SARS-CoV-2 Antigen tests for low pre-test probability indications

<u>The NPG recommends the following with regard to hospital admission screening for asymptomatic patients, either via casualty (i.e. COVID-19 not suspected clinically) or for pre-operative work-up:</u>

While the pre-test probability is low and the negative predictive value may be considered acceptable for SARS-CoV-2 antigen tests in this setting, the consequences of introducing COVID-19 in a high risk environment with a vulnerable population could be devastating if a test with lower sensitivity than the gold standard is used. For this reason, the NPG recommends that SARS-CoV-2 RT-PCR tests should be used for screening in these situations.

References

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3. Centers for Disease Control and Prevention. Interim Guidance for Antigen Testing for SARS-CoV-2 [updated 16 December 2020]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html.
4. Africa Centres for Disease Control and Prevention (Africa CDC). Interim Guidance on the use of Rapid Antigen tests for COVID-19 Response [updated 16 December 2020]. Available from: https://africacdc.org/download/interim-guidance-on-the-use-of-rapid-antigen-tests-for-covid-19-response/