Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays

Interim guidance
11 September 2020

Background

Since the beginning of the COVID-19 pandemic, laboratories have been using nucleic acid amplification tests (NAATs), such as real time reverse transcription polymerase chain reaction (rRT-PCR) assays, to detect SARS-CoV-2, the virus that causes the disease. In many countries, access to this form of testing has been challenging. The search is on to develop reliable but less expensive and faster diagnostic tests that detect antigens specific for SARS-CoV-2 infection. Antigen-detection diagnostic tests are designed to directly detect SARS-CoV-2 proteins produced by replicating virus in respiratory secretions and have been developed as both laboratory-based tests, and for near-patient use, so-called rapid diagnostic tests, or RDTs. The diagnostic development landscape is dynamic, with nearly a hundred companies developing or manufacturing rapid tests for SARS-CoV-2 antigen detection (1).

This document offers advice on the potential role of antigen-detecting RDTs (Ag-RDT) in the diagnosis of COVID-19 and the need for careful test selection. The information on Ag-RDTs in this document updates guidance that was included in the Scientific Brief entitled *WHO Advice on use of point of care immunodiagnostics test for COVID-19* published on 8 April 2020. Guidance on the use of Ag-RDTs will be regularly updated as new evidence becomes available.

Most Ag-RDTs for COVID-19 use a sandwich immunodetection method employing a simple-to-use lateral flow test format commonly employed for HIV, malaria and influenza testing. Ag-RDTs are usually comprised of a plastic cassette with sample and buffer wells, a nitrocellulose matrix strip, with a test line with bound antibody specific for conjugated target antigen-antibody complexes and a control line with bound antibody specific for conjugated-antibody. In the case of SARS-CoV-2 RDTs the target analyte is often the virus’ nucleocapsid protein, preferred because of its relative abundance. Typically, all materials that are required to perform the test, including sample collection materials, are provided in the commercial kit, with the exception of a timer.

After collecting the respiratory specimen and applying it to the test strip, results are read by the operator within 10 to 30 minutes with or without the aid of a reader instrument. The use of a reader standardizes interpretation of test results, reducing variance in assay interpretation by different operators, but requires ancillary equipment. Most of the currently manufactured tests require nasal or nasopharyngeal swab samples, but companies are carrying out studies to assess the performance of their tests using alternative sample types such as saliva, oral fluid and sample collection systems to potentially expand options for use and to facilitate safe and efficient testing. Generally, the ease-of-use and rapid turnaround time of Ag-RDTs offers the potential to expand access to testing and decrease delays in diagnosis by shifting to decentralized testing of patients with early symptoms. The trade-off for simplicity of operation of Ag-RDTs is a decrease in sensitivity compared to NAAT. Very few of the SARS-CoV-2 Ag-RDTs have undergone stringent regulatory review. Only four tests have received United States Food and Drug Administration (FDA) Emergency Use Authorization (EUA), and another two tests have been approved by Japan’s Pharmaceutical and Medical Devices Agency. Only three companies have submitted documents toward WHO’s Emergency Use Listing (EUL) procedure (2, 3).

Data on the sensitivity and specificity of currently available Ag-RDTs for SARS-CoV-2 have been derived from studies that vary in design and in the test brands being evaluated. They have shown that sensitivity compared to NAAT in samples from upper respiratory tract (nasal or nasopharyngeal swabs) appears to be highly variable, ranging from 0-94% (4-13) but specificity is consistently reported to be high (>97%). Although more evidence is needed on real-world performance and operational aspects, Ag-RDTs are most likely to perform well in patients with high viral loads (Ct values ≤25 or >10^6 genomic virus copies/mL) which usually appear in the pre-symptomatic (1-3 days before symptom onset) and early symptomatic phases of the illness (within the first 5-7 days of illness) (14, 15, 21). This offers the opportunity for early diagnosis and interruption of transmission through targeted isolation...
and cohorting of the most infectious cases and their close contacts (16). Patients who present more than 5-7 days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false negative results with Ag-RDTs is higher.

Despite these expected limitations in performance, if correctly performed and interpreted, Ag-RDTs could play a significant role in guiding patient management, public health decision making and in surveillance of COVID-19. At minimum, Ag-RDTs would need to correctly identify significantly more cases than they would miss (sensitivity ≥80%) and have very high specificity (≥97-100%). Based on these performance parameters, this interim guidance proposes several potential roles for Ag-RDT and offers general recommendations for selection of tests and key considerations for their implementation.

General recommendations for the use of SARS-CoV-2 Ag-RDTs

1. SARS-CoV-2 Ag-RDTs that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity compared to a NAAT reference assay can be used to diagnose SARS-CoV-2 infection in a range of settings where NAAT is unavailable or where prolonged turnaround times preclude clinical utility.

To optimize performance, testing with Ag-RDT should be conducted by trained operators in strict accordance with the manufacturer’s instructions and within the first 5-7 days following the onset of symptoms.

2. Appropriate scenarios for use of COVID-19 Ag-RDTs include the following:

i) To respond to suspected outbreaks of COVID-19 in remote settings, institutions and semi-closed communities where NAAT is not immediately available. Positive Ag-RDT results from multiple suspects is highly suggestive of a COVID-19 outbreak and would allow for early implementation of infection control measures. Where possible, all samples giving positive Ag-RDT results (or at least a subset) should be transported to laboratories with NAAT capability for confirmatory testing.

ii) To support outbreak investigations (e.g. in closed or semi-closed groups including schools, care-homes, cruise ships, prisons, work-places and dormitories, etc.) In NAAT-confirmed COVID-19 outbreaks, Ag-RDTs could be used to screen at-risk individuals and rapidly isolate positive cases (and initiate other

3. For initial introduction of Ag-RDTs into clinical use, countries should consider selecting some settings where NAAT confirmatory testing is currently available so that staff can gain confidence in the assays, confirm performance of the selected RDT, and troubleshoot any implementation issues encountered. Wherever NAAT will be used for confirmatory testing in patients screened using an Ag-RDT, the samples for the two tests should be collected at roughly the same time, or at most within a period of less than 2 days.

4. In situations where confirmatory testing with NAAT is not feasible, any indications that results may be incorrect should raise suspicions about validity. Examples would include patients who are test-positive but have a clinical syndrome not consistent with COVID-19, or patients with a positive test detected in a low-prevalence setting (where the predictive value of a positive test is low and the risk of false-positives high).

v) Testing of asymptomatic contacts of cases may be considered even if the Ag-RDT is not specifically authorized for this use, since asymptomatic cases have been demonstrated to have viral loads similar to symptomatic cases (17), though in that situation, a negative Ag-RDT should not remove a contact from quarantine requirements.

-2-

1 Based on well-designed and executed evaluations in representative populations
2 Risk of false positive results is high in low prevalence settings; positive predictive value is 78% if prevalence is 10% and
Other warning signals might include patients who are test-negative but have a classical syndrome, are close contacts of a case or are tested in a high-prevalence setting. In such situations, considerations should be given to repeating the test, especially if there is also any uncertainty about the visual result (faint bands) or adequacy of sampling.

5. Use of Ag-RDTs is not recommended in settings or populations with low expected prevalence of disease (e.g. screening at points of entry, blood donation, elective surgery), especially where confirmatory testing by NAAT is not readily available. Such use will not be possible until there are more data from high-quality studies confirming high specificity (>99%) of one or more of the commercialized Ag-RDT test kits.

Selection of tests for procurement and implementation:

Though there are a limited number of SARS-CoV-2 Ag-RDTs currently available commercially, multiple products, of variable quality and performance, are expected to enter the marketplace soon. As noted in the Introduction, most commercial SARS-CoV-2 Ag-RDTs use a conventional lateral flow format with colloidal gold or other visible dye as indicators. Several systems, including some with US FDA approval under EUA, use alternative indicators that may lead to enhanced sensitivity but require a specific device to read and interpret the test results.

There are a number of factors to consider when selecting Ag-RDTs for use in the scenarios presented above, in the recommendations section. These include:

1. Quality of available data used to validate the test. The source of data should be considered (independent vs. internal/corporate sponsorship) as should study design (e.g. the reference standard used, the type of specimen, the delay between sample collection and test execution and the number of days since symptom onset), the number of subjects enrolled, and the setting of enrolment. As the concentration of virus in specimens is the greatest predictor of test sensitivity, the selection of patients and study sites is of critical importance. Prospective clinical studies are generally superior to retrospective studies. Data from studies independent of corporate sponsorship have particular value if the studies are well-performed.

2. Reported performance. Data demonstrating the performance of an RDT should be carefully reviewed before procurement is initiated. Given the relatively low prevalence of active SARS-CoV-2 infections even in settings with community transmission, high specificity (minimum ≥97% and ideally ≥99%) is necessary to avoid many false-positive results. Sensitivity will depend on the status of patients studied (degree of illness, days since onset of symptoms, etc.) as well as the product quality, but should reach a minimum of ≥80%. A useful assessment is the sensitivity of the test in patients with a rRT-PCR cycle threshold (Ct) below a specific value (e.g. 28 or 30), because the virus is expected to be abundant in respiratory samples when the test is in this range, and test sensitivity correspondingly high (exceeding 90% in some published and unpublished studies) (4,11). It is important to note, however, that Ct values at a given input concentration of target RNA vary between rRT-PCR assays and are not strictly quantitative.

3. Manufacturing quality and regulatory status. Tests should be procured from manufacturers who work under a quality management system (e.g. ISO 13485) and with at least local regulatory approval or right of free sale granted by the country of manufacture. RDTs, as all in vitro diagnostics intended for clinical use, should undergo a rigorous and transparent regulatory review. Approval or authorization by a stringent regulatory body and/or Emergency Use Listing by WHO should be available at the time of procurement.

4. Manufacturing capacity and further evidence of quality. Many new companies without a history of success in the manufacture, sales and support of in vitro diagnostics are entering the market with SARS-CoV-2 Ag-RDTs. Procurers should consider the range of other products offered by the company (especially lateral flow tests), what regulatory approvals they have for non-emergency diagnostic products, and their manufacturing and post-market surveillance capacity. Many companies are able to manufacture high-quality prototypes or completed tests at low volume but may have difficulty when scaling up manufacturing to meet global needs.

5. Distribution and technical support. Consideration should be given to a supplier’s distribution and product support capacity, especially in low and middle-income countries. This is particularly true for tests that require additional equipment like readers.

6. Shipping and storage conditions and shelf-life. The capacity to withstand temperature stress and having an extended shelf-life are critical to the ease-of-use of Ag-RDTs. With new products, shelf-life must be estimated based on accelerated stability studies (usually at higher temperatures), but target shelf-life should be at least 12-18 months at 30°C and...
Ideally 40°C. A cold chain requirement for shipping and/or storage would significantly increase the cost and complexity of procurement and distribution.

7. **Specimen collection requirements.** SARS-CoV-2 Ag-RDTs vary in their requirements for specimen type, number of processing steps, need for accurate timing, instrumentation and interpretation of results, which will influence the extent of training and supervision required. For this reason, an ease-of-use assessment is an important consideration along with test performance.

8. **Contents of test kit.** Standard kit contents do not necessarily include everything required to perform and quality control the test, and this must be verified prior to purchase. Several commercially available Ag-RDTs for SARS-CoV-2 utilize a reading instrument.

9. **The cost of the test.** The cost of tests will vary according to the test and the volume to be purchased. In general, they should be less expensive than PCR tests. The cost of transportation, import tariffs, storage, end-user training (and supervision) and post-purchase quality control testing activities required to support quality implementation of RDTs must also be considered.

10. **Availability, completeness and clarity of instructions for use.** These should be clear, contain illustrations and be user-friendly for a non-laboratory specialist.

**Implementation considerations:**

1. Even though Ag-RDTs may be considerably easier to perform than NAAT, they still require that supplier-recommended procedures be strictly followed with due attention to documentation, execution of time-dependent or volume-dependent steps, storage conditions and shelf-life and equipment and stock management. All test operators must have training in sample collection, relevant biosafety, performance of the test and interpretation and reporting of results as well as in waste management. Quality control measures also need to be put in place.

2. Post-market surveillance, with regulatory oversight, is critical to discover defects in product performance and is an important requirement for the manufacturer. The health system should ensure there is monitoring and evaluation of COVID-19 diagnostic testing activities and clear mechanisms for reporting problems (18).

3. Use of instrumented detection systems demands additional training requirements (instrument use, calibration as required, service requirements, operating conditions) and sufficient infrastructure, such as a reliable source of electricity.

4. Sample collection is one of the most critical factors affecting performance of Ag-RDTs. Instructions for use should be carefully followed, and any staff collecting samples should be trained in the methodology.

5. Each of these tests has a specifically indicated method for sample processing after collection. Instructions should be followed precisely, and no alternative reagents used (e.g. water or other liquid instead of dilution/mixing buffer).

6. Biosafety requirements for operators must be in place – personal protective equipment, biohazard waste bag and good ventilation are essential (19).

**Methods**

This Interim Guidance document outlines potential use and non-use case scenarios for SARS-CoV-2 antigen-detecting RDTs based on minimum performance criteria. Minimum performance requirements for Ag-RDTs were established through a formal process of target product profile (TPPs) development for priority SARS-CoV-2 diagnostics (20). They were informed by an evolving understanding of the temporal dynamics of viral shedding and transmissibility and the anticipated benefits of earlier and expanded testing. PubMed and medRxiv databases were searched for both peer-reviewed and published, pre-print reports of test accuracy of point of care/near patient rapid antigen-detecting SARS-CoV-2 tests. One systematic review of diagnostic test accuracy was identified (21). Additionally, unpublished independent reports on the performance of two SARS-CoV-2 Ag-RDTs were shared confidentially with WHO. Interim guidance was reviewed by members of the WHO Reference Laboratory Network for COVID-19 and members of the WHO COVID-19 Diagnostics Target Product Profile Review Group, as well as other outside experts.

We recognize the shortcomings of the available evidence. They include small sample sizes, skewed sampling based on expected presence or absence of SARS-CoV-2 infection, and lack of details in studies aimed at validating tests regarding symptom status or time from symptom onset. In addition, the lack of data from asymptomatic cases, use of tests outside of manufacturers’ instructions for use and performance of tests in laboratories as opposed to point of care/near-patient settings limit the generalizability of recommendations. Nonetheless, it was concluded that some Ag-RDTs are likely to at least meet and likely exceed minimum performance requirements in the early
phase of the illness (within the first 5-7 days, when viral loads and risk of transmission are highest). Expanding testing to potentially interrupt transmission through the use of antigen RDTs is considered more beneficial than not testing or performing tests that fail to inform infection control measures due to very long turnaround times or the risk of false negatives in patients with low viral loads.

Test performance

The performance of an Ag-RDT is determined by the sensitivity and specificity of the test to detect a SARS-CoV-2 infection compared with a reference standard, NAAT (generally rRT-PCR).

**Sensitivity** is the percentage of cases positive by a NAAT reference standard that are detected as positive by the Ag-RDT under evaluation.

**Specificity** is the percentage of cases negative by a NAAT reference standard that are detected as negative by the Ag-RDT under evaluation. The prevalence of disease in the community being tested strongly affects the predictive value of a positive or negative result (see Annex 1). Thus, the clinical value of a positive or negative test result will depend on what action is taken on the basis of the test result when interpreted in the context of local prevalence.

In general, the higher the prevalence of SARS-CoV-2 infection in the tested population, the more likely a person who tests positive is to have COVID-19. The lower the prevalence in the community, the more likely a test-negative patient is not to have the disease, see Annex 1. For example, when the prevalence of active SARS-CoV-2 infection in a community is 1%, even a test that is 99% specific would have a poor positive predictive value, since one-half of all positive results would be false positive.

Roles for antigen detecting RDTs for case management and surveillance for COVID-19

Use of Ag-RDTs can be considered in countries or areas that are experiencing widespread community transmission, where the health system may be overburdened and where it may not be possible to test all or any suspect cases by NAAT. As with all diagnostic tests, but especially those with sub-optimal sensitivity and/or specificity, to correctly interpret and act on the results of the RDT, the prevalence of disease (according to the reference standard) must be estimated based on surveillance, since this determines the positive and negative predictive values (PPV and NPV, respectively) of the RDTs (Annex 1). The proposed process for utilizing an Ag-RDT for COVID-19 case management when there is widespread community transmission is shown in Figure 1. In such a setting, the pre-test probability of COVID-19 disease (the likelihood that the patient has COVID-19 before their results are known, based on epidemiologic and clinical factors) is relatively high, and positive test results have a high predictive value. Likewise, in a setting of community transmission, the predictive value of a negative RDT result may be low, even when there are strong epidemiologic or clinical indicators of COVID-19 exposure or disease.

NPV- negative predictive value; PPV – positive predictive value
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Table 1. Situations where SARS-CoV-2 Ag-RDTs should not be used, based on currently available information

<table>
<thead>
<tr>
<th>Do not use SARS-CoV-2 Ag-RDTs:</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>In individuals without symptoms unless the person is a contact of a confirmed case</td>
<td>Pre-test probability (the likelihood, before testing, that the patient has the disease based on epidemiology, case contact, clinical findings) is low.</td>
</tr>
<tr>
<td>Where there are zero or only sporadic cases</td>
<td>Ag-RDTs are not recommended for routine surveillance purposes or case management in this setting. Positive test results would likely be false positives. Molecular testing is preferred.</td>
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<tr>
<td>Appropriate biosafety and infection prevention and control measures (IPC) are lacking</td>
<td>To safeguard health workers, respiratory sample collection for any test from patients with suspected COVID-19 requires that operators wear gloves, gown, mask and face shield or goggles (19, 22, 23).</td>
</tr>
<tr>
<td>Management of the patient does not change based on the result of the test</td>
<td>If test-positive and test-negative patients will be treated the same way because of unknown or low PPV and/or NPV, then there is no benefit to testing.</td>
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<td>For airport or border screening at points of entry</td>
<td>Prevalence of COVID-19 will be highly variable among travellers, and it is therefore not possible to determine PPV and NPV of test results. Positive and negative tests would require confirmatory testing to increase PPV and NPV for decision making.</td>
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<tr>
<td>In screening prior to blood donation</td>
<td>A positive RDT result would not necessarily correlate with presence of viremia. Asymptomatic blood donors do not meet the definition of a suspect case (24).</td>
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</table>

Factors influencing test performance

As mentioned above, many factors may affect the performance of antigen-detecting RDTs. Consequently, findings in clinical settings may be variable. The following should be taken into account:

- patient factors such as the time from illness onset and immune status
- sample type (upper or lower respiratory tract), quality and processing, including storage conditions and dilution in viral transport medium
- viral factors including the concentration and duration of viral antigen shedding and structural variation in the target antigen, cross reactivity with other viruses
- specific protein target, as some antigens are produced in higher concentrations than others, e.g. nucleocapsid versus spike proteins
- product design or quality issues including:
  - insufficient antibody quantity or affinity for the target antigen(s)
  - poor packaging and exposure to heat and humidity during improper transport and/or storage, which can degrade antibodies in the test
  - unclear or incorrect instructions that can affect test performance
  - inadequate training or competency of the test operator, which may lead to error in preparing the antigen-detecting RDT, performing the test or interpreting the result, with erroneous conclusions.

Future updates and product specific recommendations

WHO is working closely with groups evaluating the performance and operational characteristics of commercialized SARS-CoV-2 antigen detecting RDTs to systematically compile the evidence as it emerges and coordinate updates. Currently, there is insufficient evidence on performance and operational use to recommend specific commercial products.
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References


Acknowledgments

This document was developed in consultation with:

External: Sergio Carmona, FIND, Switzerland; Arlene Chua, Médecins Sans Frontières, Switzerland; Antonino Di Caro, Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani, Italy; Sally Hojvat, Partners in Diagnostics, USA; Erik Karlsson, Institut Pasteur du Cambodge, Cambodia; Rosanna Peeling, London School of Hygiene & Tropical Medicine, UK; Leo Poon, Hong Kong University, China, Hong Kong SAR; Chantal Reusken, RIVM, Netherlands; Bill Rodriguez, Draper Richards, Kaplan Foundation, USA; Juliane Sacks, FIND, Switzerland; Anne von Gottberg, National Institute for Communicable Diseases, South Africa


Declaration of interests

Declarations of interests were collected from all external contributors and assessed for any conflicts of interest. There were no significant conflicts of interest declared.

WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance document will expire 2 years after the date of publication.

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WHO reference number: WHO/2019-nCoV/Antigen_Detection/2020.1
Annex 1

Annex : Positive predictive value (PPV) and negative predictive value (NPV) and the number of true positive (TP), false positive (FP), true negative (TN) and false negative (FN) tests in a population of 100 000 with the prevalence of COVID-19 estimated at 5, 10, 20, 30% prevalence and based on recommended performance criteria: sensitivity of 70, 80%, 90% and specificity of 97.5% and 99.5%.

<table>
<thead>
<tr>
<th>Example prevalence target populations</th>
<th>Prevalence (%)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
<th>TP</th>
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<th>No. with disease</th>
<th>No. positive tests</th>
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