Antigen-detection in the diagnosis of SARS-CoV-2 infection

Interim guidance

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World Health Organization

Key Points

- Diagnostic testing for SARS-CoV-2 is a critical component to the overall prevention and control strategy for COVID-19.
- Tests should be reliable, affordable, accessible and provide results rapidly to ensure appropriate clinical care and support for patients and inform actions to prevent onward spread of SARS-CoV-2.
- Antigen-detecting diagnostic testing uses upper respiratory specimens or saliva to test for SARS-CoV-2 infection by detecting viral proteins (e.g., nucleoprotein).
- Antigen-detecting rapid diagnostic tests (Ag-RDTs) can offer a faster and less expensive way to diagnose active SARS-CoV-2 infection than nucleic acid amplification tests (NAATs).
- Ag-RDTs perform best in individuals with high viral load, early in the course of infection, and will be most reliable in settings were SARS-CoV-2 prevalence is ≥ 5%. When there is no transmission or low transmission, the positive predictive value of Ag-RDTs will be low, and in such settings NAATs are preferable for first-line testing or for confirmation of Ag-RDT positive results.
- WHO recommends the use of Ag-RDTs that meet minimum performance requirements of ≥ 80% sensitivity and ≥ 97% specificity. Ag-RDTs are less sensitive than NAAT, particularly in asymptomatic populations, but careful selection of cohorts for testing can mitigate this limitation.
- Ag-RDTs should be prioritized for use in symptomatic individuals meeting the case definition for COVID-19, and to test asymptomatic individuals at high risk of infection, including contacts and health workers, particularly in settings where NAAT testing capacity is limited.
- Positive Ag-RDT results from multiple suspected cases is highly suggestive of a COVID-19 outbreak.
- Ag-RDT can be used outside of clinical and laboratory settings, including in communities. Ag-RDTs should be performed by trained operators in accordance with instructions and adherence to storage and operational temperature requirements.
- WHO recommends that Ag-RDTs meeting minimum performance requirements can be used for primary case detection, contact tracing, during outbreak investigations and to monitor trends of disease incidence in communities.
- Sample collection is one of the most critical factors affecting the performance of any diagnostic test on respiratory fluids, including Ag-RDTs, and post market surveillance to monitor and evaluate tests should be in place.

Background

Diagnostic testing for SARS-CoV-2 is a critical component to the overall prevention and control strategy for COVID-19. Countries should have a national testing strategy in place with clear objectives that can be adapted according to changes in the epidemiological situation, available resources and tools, and country-specific context. It is critical that all SARS-CoV-2 testing is linked to public health actions to ensure appropriate clinical care and support and to carry out contact tracing to break chains of transmission.

Since the early days of the SARS-CoV-2 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 COVID-19. Since mid-2020, less expensive and faster diagnostic tests that detect antigens specific for SARS-CoV-2 infection have become commercially available, and several have achieved WHO Emergency use listing.

Antigen-detecting diagnostic tests are designed to directly identify SARS-CoV-2 proteins produced by replicating virus in respiratory secretions (or oral fluid/saliva) and have been developed as both laboratory-based tests and rapid diagnostic tests (RDTs) intended for near-patient use. The diagnostic development landscape is dynamic, with over two hundred tests for SARS-CoV-2 antigen detection on the market, of which 85% can be delivered at the point of care and the other 15% for use on high throughput machines in laboratory-based settings (1).

Purpose of this document

This interim guidance offers general recommendations for selection of antigen-detecting rapid diagnostic tests (Ag-RDTs) and key considerations for their implementation.

Changes from the previous version

In September 2020, WHO released its first interim guidance on the potential role of Ag-RDTs in the diagnosis of COVID-19 (Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays), which stressed the need for careful test selection. This document has been updated to incorporate new findings concerning test performance across Ag-RDT brands and sample types.

The document also provides guidance about the use of Ag-RDTs in specific populations and settings, including asymptomatic health workers and long-term care facility workers. It additionally provides more detailed recommendations on product selection and storage, including precautions about the potential for brief periods of storage at temperatures that are too high or too low to negatively affect Ag-RDT performance.

Process and methods

The recommendations in this document are based on minimum performance requirements for Ag-RDTs (≥ 80% sensitivity and ≥ 97% specificity) compared to a nucleic acid amplification test in suspected COVID-19 cases. These standards were established through a formal process of target product profile (TPPs) development for priority SARS-CoV-2 diagnostics (2,3). They were further informed by an evolving understanding of the temporal dynamics of SARS-CoV-2 shedding and transmissibility and the anticipated benefits of earlier and expanded testing. These target performance parameters have been shown to be achievable mainly in symptomatic test populations and by some Ag-RDTs on the market.

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. Two systematic reviews of diagnostic test accuracy were identified (4,5). Additionally, independent reports coordinated by FIND and reports listed on the Universitäts Klinikum Diagnostics Global Health site were used to identify publications after the cut-off point of the last systematic review (30 April 2021) up until 10 May 2021, with a special focus on diagnostic test accuracy in asymptomatic populations.

Other WHO guidance documents were reviewed for recommendations on testing in specific populations including health workers, contacts of COVID-19 cases, workplaces, schools and travellers.

This 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.

Limitations

The number of tests examined in published reports is still limited relative to the hundreds of test brands available on the market. Performance estimates should be cautiously interpreted in the context of their methodological limitations and the settings in which they were conducted. More direct comparisons of test brands are needed, as well as data on performance in clearly defined cohorts of asymptomatic people, and by different operators, including self-testing. Although more studies are being conducted according to the manufacturer's instructions and in point of care/near-patient settings, there is still room for improvement.

More controlled studies are needed on the cost, operational effectiveness and impact of various screening strategies to support the development of additional recommendations.

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

In all settings, the **first priority of COVID-19 control is to deploy available financial and human resources toward the prompt identification of SARS-CoV-2 in symptomatic individuals and contacts of confirmed or probable cases and enable them to be compliant with countermeasures including isolation**. If correctly performed and interpreted, Ag-RDTs can play a significant role in this effort and may be more cost effective than NAAT in symptomatic populations (6).

Notwithstanding variations in test performance, antigen-based diagnosis offers the opportunity for timely diagnosis and interruption of transmission if coupled with targeted, rapid isolation and cohorting of the most infectious cases and their close contacts (7). 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 (5). Targeted expansion of testing to potentially interrupt transmission through the use of Ag-RDTs is considered more beneficial than not testing or performing tests that fail to inform infection control measures due to the prolonged turnaround times sometimes associated with NAAT.

The technology used for SARS-CoV-2 Ag-RDTs has been described in detail in the WHO September 2020 interim guidance document. 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. The trade-off for simplicity of operation of Ag-RDTs is a decrease in sensitivity and specificity compared to NAAT (4). However, as some Ag-RDTs have been shown to consistently detect SARS-CoV-2 in those samples containing levels of viral nucleic acid associated with positive viral cultures (~10E6 RNA copies/mL), Ag-RDTs may be detecting the majority of infectious cases despite a significantly lower analytic sensitivity than NAAT (8,9). Transmission from individuals with viral loads below this viral culture threshold can still occur, particularly in certain social and behavioral contexts (10,11). Nonetheless, the ability of Ag-RDTs to rapidly detect the most infectious SARS-CoV-2 cases in settings without rapid access to NAAT is likely to have a positive impact on disease control.

Who can use Ag-RDTs ?

To optimize performance, testing with Ag-RDTs should be conducted by trained operators in strict accordance with the manufacturer's instructions. Several organizations and institutions including WHO and FIND have developed comprehensive training materials for SARS-CoV-2 Ag-RDTs. Criteria for operator eligibility should be in accordance with national laws and regulation on use of vitro diagnostic tests.

When to use Ag-RDTs ?

The results of Ag-RDTs will be most reliable in areas when there is ongoing community transmission (≥5% test positivity rate). (See the Annex.)

When there is no transmission or low transmission, the positive predictive value [1] of Ag-RDTs will be low (many false positives), and in this setting NAAT is preferable as the first-line testing method or for confirmation of positive Ag-RDTs.

Where to use Ag-RDTs ?

Ag-RDTs do not require a laboratory and may be performed by trained operators in any setting where appropriate biosafety measures and storage conditions are ensured. **It is critical, however, that Ag-RDT results be registered for local use and that diagnosed cases be reported through the local reporting mechanisms including the laboratory network reporting system and/or relevant national surveillance systems**.

Who should be tested with Ag-RDTs?

Population: Symptomatic individuals (suspected COVID-19 cases) in the first 5-7 days since onset of symptoms

WHO recommends that SARS-CoV-2 Ag-RDTs that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity compared to a NAAT reference assay [2] can be used to diagnose SARS-CoV-2 in suspected COVID-19 cases. Clinical discretion considering epidemiological context, clinical history and presentation and available testing resources should determine if negative Ag-RDT results require confirmatory testing with NAAT or repeat testing with Ag-RDTs (within 48hrs) if NAAT is not readily available (Figure 1). Note that the safe management of

[1] Positive predictive value (PPV) is the probability that patients with a positive test result have the disease. At 0.1% prevalence, a test with 98% specificity would have a PPV of 4%, meaning that 96 out of 100 positive results would be false positives.

[2] Based on well-designed and executed evaluations in representative populations

patients with Ag-RDT-positive and negative results will depend on the test's performance and the community prevalence of SARS-CoV-2. The prevalence of infection (according to the reference standard) must be estimated based on surveillance**,** since this influences the positive and negative predictive values (PPV and NPV, respectively). (See Annex 1.)

Rationale

Transmissibility of the virus depends on the amount of viable virus being shed and expelled by a person, the type of contact they have with others, the setting and what infection prevention and control (IPC) measures are in place. SARS-CoV-2 infections can be symptomatic or asymptomatic and both symptomatic and asymptomatic infected persons can transmit SARS-CoV-2.

Available published data suggest that infected individuals 2-3 days prior to onset of symptoms and first 5-7 days of illness have the highest viral loads and therefore are most likely to contribute to onward transmission (12). Many Ag-RDTs can detect > 90% of cases with the high viral loads e.g. Ct < 25-30 seen in these early days following onset of symptoms.

One systematic review of 79 studies found that 20% (17–25%) of people remained asymptomatic throughout the course of infection (13). Studies suggest that asymptomatic individuals who are infected are less likely to transmit the virus than those who develop symptoms(14), (15). One meta-analysis estimated that there is a 42% lower relative risk of asymptomatic transmission compared to symptomatic transmission (16).

Populations and rationale: Asymptomatic Individuals

Levels of virus in asymptomatic or pre-symptomatic cases can be similar to symptomatic cases and therefore, asymptomatic individuals can transmit to others (11,17).

A number of studies have compared NAAT and Ag-RDTs in asymptomatic populations that varied in their risk profiles and represented heterogeneous viral trajectories. As might be expected, NAAT performed significantly better than Ag-RDTs (18), (19), (20), (21), (22), (23), (24). In these contexts Ag-RDTs often do not meet WHO's recommended performance characteristics. This is not always the case in more homogenous groups of contacts of cases tested within the COVID-19 incubation period (25–28).

The prevalence of SARS-CoV-2 infection in most asymptomatic populations is low. Consequently, even if the Ag-RDT specificity is very high, false positive test results will be more likely than true positives (i.e., there will be a low PPV, see the Annex). Repeat Ag-RDT testing, or confirmatory testing with NAAT will be required to avoid unnecessary isolation. This general rule also applies to health care settings, where patients with false positive Ag-RDT results should not be isolated alongside those with true-positive test results.

Therefore WHO recommends that use of Ag-RDTs among asymptomatic populations be limited to contacts of confirmed or probable cases and to at-risk health workers until more evidence is available on the benefits and cost effectiveness of testing low-risk groups with no known exposure to SARS-CoV-2, particularly in settings where testing capacity is limited. More details are provided below.

Asymptomatic Contacts of confirmed COVID-19 cases

Several studies report Ag-RDT performance that approaches or meets WHO recommendations among symptomatic and asymptomatic contacts of cases (25,26,29,30).

WHO therefore continues to recommend that Ag-RDTs can be used to screen for SARS-CoV-infection in contacts of cases, particularly those who are at a higher risk of developing severe disease and /or have had high levels of exposure to SARS-CoV-2 (31).

The need for confirmatory testing of positive Ag-RDT results should be based on incidence of infection in the community (including circulation of variants of concern), immunity status (past infection or vaccination) and availability of NAAT. (See Figure 1).

Health workers[3]

WHO recommends early detection of SARS-CoV-2 infection among health workers through syndromic surveillance and/or regular testing (32). Acute care health workers who work in COVID-19 services or facilities have the highest priority, followed by health workers prioritized by risk in other clinical areas. Clear intervals for routine testing or time points have not been identified and should be adjusted according to prevalence (33–35). More frequent testing will have obvious cost implications and variable yield depending on the transmission intensity, exposure risk and compliance with the testing strategy (36–38).

WHO recommends routine testing, if feasible, for health workers in long-term care facilities. At minimum, testing should be done as soon as a positive case of COVID-19 is identified in either residents or staff and weekly, thereafter, if resources allow, until there are no

[3] Health workers are defined by WHO as all people engaged in actions with the primary intent of enhancing health, including social care workers who often have roles in the provision of care in long-term care facilities and in community settings.

cases of COVID-19 in the facility. Visitors should also be screened prior to visits to long-term care facilities (39).

The majority of studies screening health workers have employed NAAT, not Ag-RDTs. One pilot study in Slovenia suggested Ag-RDTs were not of sufficient sensitivity to identify infections among asymptomatic health workers (40). However, modeling exercises (not yet supported by human studies) suggest that what Ag-RDTs lack in sensitivity might be offset through serial testing in the early stages of infection to identify asymptomatic cases and help interrupt SARS-CoV-2 transmission (41). Ag-RDTs have clear advantages for health workers because the decentralized testing and rapid results leads to more rapid isolation after a positive result.

Population and Rationale: Suspected COVID-19 cases in outbreak investigations

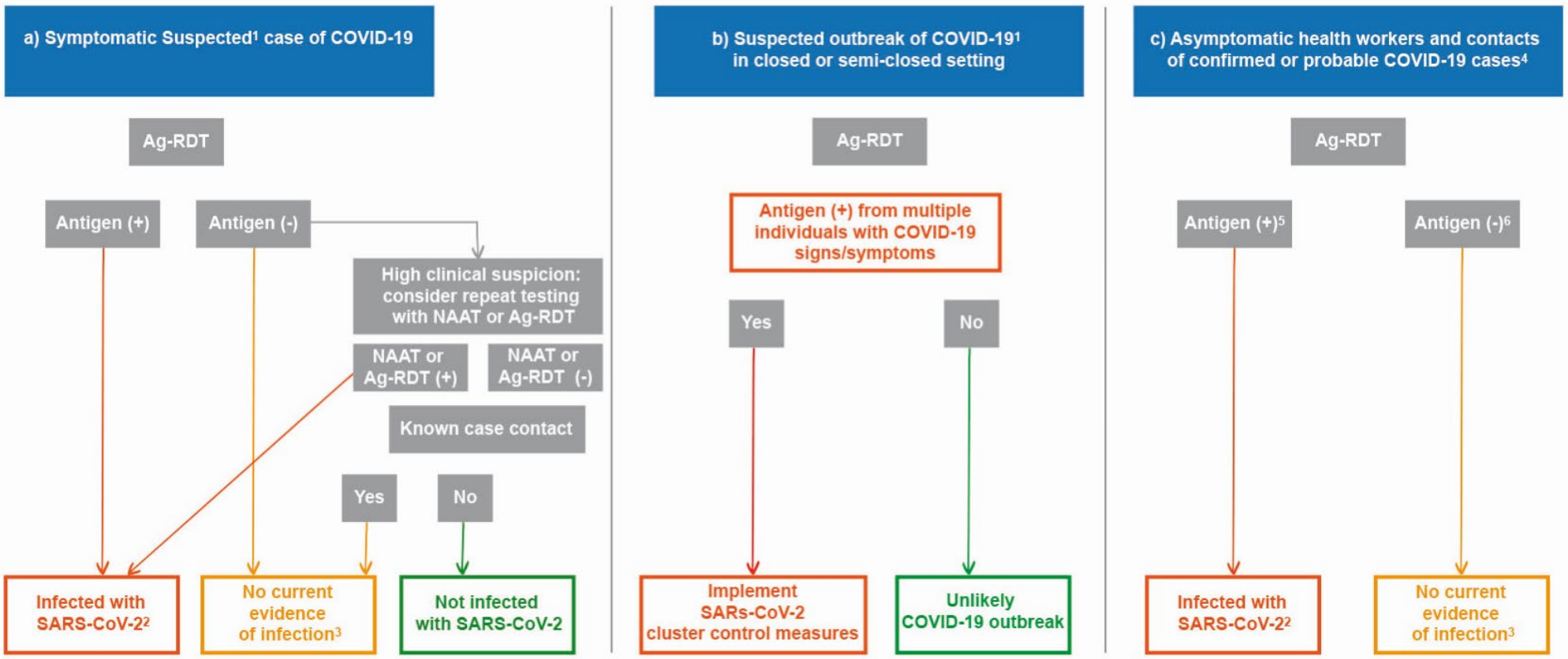
Because of their ease of use and rapid turnaround time, Ag-RDTs are a useful tool to quickly identify a cluster or outbreak and support the investigation and implementation of public health interventions to control transmission. The finding of positive Ag-RDT results from multiple individuals is highly suggestive of a COVID-19 outbreak and would support early implementation of appropriate infection control measures and case management. (Figure 1).

Summary recommendations for priority Ag-RDT use

Ag-RDT testing is recommended in settings likely to have the most impact on early detection of cases for care and contact tracing and where test results are most likely to be correct. Priority uses are indicated in Figure 1 and include:

a. **Community testing of symptomatic individuals meeting the case definition of suspected COVID-19.** Individuals with positive Ag-RDT results should be rapidly isolated and contact tracing efforts initiated. The field sensitivity of Ag-RDTs, especially when testing lightly symptomatic cohorts or mixing sample collection methods, may be significantly lower that demonstrated in controlled trial settings, missing 25-50% of infections compared with NAAT. Symptomatic individuals who are Ag-RDT-negative but at high risk should be considered for retesting with NAAT (42) where accessible (results in <24 hours) or with Ag-RDT if not.
b. **To detect and respond to suspected outbreaks of COVID-19** including in remote settings, institutions and semi-closed communities (e.g. schools, care-homes, cruise ships, prisons, workplaces and dormitories), especially where NAAT is not immediately available.
c. **To screen asymptomatic individuals at high risk of COVID-19**, including health workers, contacts of cases and other at-risk individuals.

Figure 1: SARS-CoV-2 Antigen RDT Algorithm*



* The results of Ag-RDTs will be most reliable in areas where there is ongoing community transmission (≥5% test positivity rate)

Ag = antigen, NAAT = nucleic acid amplification test.

1. WHO defintions of COVID-19 suspected case are found here; national guideline definitions may vary.
2 Case registration, isolation and contact tracing are necessary for all detected cases. (43–45).
3. Quarantine is necessary for contacts of confirmed or probable cases. If symptoms develop suspects should be tested as per a).
4.WHO defines contacts here and confirmed and probable cases here.
5. In instances of lower pretest probability, such as low incidence of SARS-CoV-2 infection in the community, clinical discretion should determine if positive Ag-RDT results need confirmation by NAAT.
6. For health workers and long-term care facility workers serial Ag-RDT testing (at least weekly) should be considered where NAAT testing is not readily available, especially during periods of intense community transmission (32,39).

Specific groups and applications where additional research is needed to refine the role of Ag-RDTs

Travellers

WHO recommends a thorough risk assessment as a key element of the decision-making process regarding SARS-CoV-2 testing policies for international travelers (46). International travelers should not be considered by default or by nature as suspected SARS-CoV-2 cases or contacts or as a priority group for testing, in particular when resources are limited, to avoid diverting resources from settings and patients where testing can have a higher public health impact and drive action.

Many countries and aviation operators have adopted strict testing requirements pre- and/or post-travel at points of entry to reduce the risk of importation, exportation and/or onward transmission of SARS-CoV-2 infection and to prevent movement of SARS-CoV-2, including variants of concern and interest, across borders. The public health effectiveness and impact of different testing strategies has been reviewed and continues to be investigated (47). As in any setting, testing coverage, performance and infection prevalence will have an impact on the effectiveness of testing. Because Ag-RDTs are less sensitive than NAAT, particularly in asymptomatic populations, modelling suggests they will potentially fail to detect up to half of SARS-CoV-2-infected travellers (48). This challenge could potentially be reduced with serial testing to identify individuals recently infected with SARS-CoV-2 who are incubating the disease, but evidence for this approach is lacking.

Travellers are expected to be a low-prevalence population; if countermeasures are already in place due to moderate or high community transmission, testing of international travellers is likely to have less impact. In these circumstances, the risk of false-positive results is high; and confirmatory testing with NAAT following positive Ag-RDT is strongly advised.

Workplaces

In workplaces, WHO recommends testing where there is a high risk of exposure (49).

Students attending educational institutions

A rapid scoping review was carried out to identify and map the evidence assessing the impacts of measures implemented to reopen schools or keep schools open during the current pandemic (50). It revealed that the majority of studies were based on mathematical modelling (31).

WHO currently does not recommend mass screening of students using SARS-CoV-2 diagnostic tests. However, screening for signs and symptoms of COVID-19 and prompt testing of suspected cases and tracing of contacts are recommended (51).

General population screening

There have been many publications using mathematical modelling to estimate the impact of mass testing approaches. Systematic reviews have been largely based on these modelling studies (52,53) . A small number of real-life studies have been conducted (54–56). Given the significant costs involved, the lack of evidence on impact and cost-effectiveness of such approaches and the concern that this cost-intensive approach risks diverting resources from higher priority testing indications, mass community-based testing of asymptomatic individuals is not currently recommended.

Self-testing

Because of their user-friendly characteristics, Ag-RDTs have been considered for self-testing. WHO recognizes that self-testing offers potential advantages as a complement to health system-based testing by trained providers, such as earlier and increased access to testing for those who can afford it. However, self-testing may impair countries' ability to monitor disease trends, ensure appropriate case management and identify and track variants.

There is limited data to date on performance of Ag-RDTs by untrained users guided by manufacturer's instructions for use. Some such studies demonstrate comparable accuracy to that being reported by trained users (57–59), but some show poorer sensitivity in self-testing cohorts (60). The definition of self-testing sometimes includes self-sampling, self-performance of testing, and self-reading of test results, or all three. In any case, it is important that any self-testing be carried out in alignment with required biosafety and waste management measures, and that results be reported to the appropriate health authorities.

The costs, benefits and risks must all be carefully weighed before embarking on self-testing approaches. WHO is reviewing ongoing research on self-testing and emerging evidence of its potential utility in COVID-19 control.

SARS-CoV-2 Ag-RDT Performance Characteristics

Because many factors can affect the performance of Ag-RDTs, findings in clinical settings may be variable. The following should be taken into account:

- patient factors such as the time from illness onset, symptoms and immune status
- sample type [nasopharyngeal, nasal, anterior nares, mid-turbinate, oropharyngeal (61), lower respiratory tract, saliva or oral fluid], quality and processing of samples, 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
- specific protein target detected in the assay; noting that some antigens, such as nucleocapsid, are produced in higher concentrations than others, such as spike proteins; or have higher mutation rates (spike > nucleocapsid) that may affect antibody binding
- product design or quality issues including:
    - insufficient antibody quantity or affinity for the target antigen(s)
    - potential cross reactivity with other microorganisms
    - poor packaging allowing exposure to heat and humidity, which can degrade antibodies in the test
    - unclear or incorrect instructions that can affect test performance
- improper transport and/or storage
- inadequate training or competency of the test operator, which may lead to errors in preparing the Ag-RDT, performing the test or interpreting the result.

A number of studies evaluating sensitivity and specificity of different Ag-RDTs have been published over the past eight months. Study quality is variable, the scope of brands evaluated is limited and assessments are predominantly restricted to health worker-administered testing of symptomatic populations (4), (5). The cohort of individuals tested and the quality of the operators performing the test have a considerable impact on test performance. The table below illustrates the results of a recent systematic review of instructions for use (IFU)-compliant studies [4] including symptomatic and asymptomatic subjects (4).

Table 1: Summary SARS-CoV-2 Ag-RDT performance in studies performed according to manufacturer's instructions for use

Population	Sensitivity (95%CI)	Specificity (95% CI)
All subjects	72.0% (56.5% to 83.5%)	99.2% (98.5% to 99.5%)
Symptomatic	75.1% (57.3% to 87.1%)	99.5% (98.7% to 99.8%)
Asymptomatic	48.9% (35.1% to 62.9%)	98.1% (96.3% to 99.1%)

More recent pre-prints and publications of test performance in a variety of settings and populations including community screening, pregnant women, and children confirm this lower performance in comparison to NAAT (5), (19), (20), (21), (22), (23), (18), (62).

Among asymptomatic contacts of confirmed cases tested several days after exposure, however, Ag-RDTs showed performance comparable with that seen in symptomatic cases but lower than that seen with NAAT (25–28). This is not unexpected based on described viral load kinetics (11,17).

Ag-RDTs perform best in individuals with high viral loads (Ct values ≤25-30, ~10E5/6 RNA copies/mL) 1-3 days prior to onset of symptoms and during the first 5-7 days of illness (17). In the most recent Cochrane systematic review, overall sensitivity in those with higher viral load (Ct ≤ 25) was 94.5% (compared to 40.7% in those with lower viral load). (4). According to Brummer et al., the highest Ag-RDT sensitivity was found with upper-respiratory swab samples (75.5% for anterior nasal or mid-turbinate and 71.6% for nasopharyngeal sampling) in comparison to other sample types (5).

The two systematic reviews of Ag-RDT performance identified in the preparation of this guidance document revealed high specificity. The overall specificity in IFU-compliant studies was 99.6%, (4) and pooled specificity of 99.0% for all but two tests (5). Specificity was not affected by the presence or absence of symptoms.

Considerations for product selection

As noted previously, the minimum performance requirements for Ag-RDTs are that they have sensitivity ≥80%, and high specificity (≥97-100%). Most Ag-RDTs use a conventional lateral flow format with colloidal gold or other visible dye as indicators. Several systems, including some with United States Food and Drug Administration approval under Emergency Use Authorization and in the WHO Emergency Use Listing pipeline, require a specific device to read and interpret the test results.

There are a number of factors to consider when selecting Ag-RDTs. These include the following.

1. **Quality of available data used to validate the test**. The source of data should be considered (independent vs commercially managed or funded) 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. Considering that 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

[4] Instructions for use 'compliance' considers sample type, use of viral transport media and timing from sample collection to testing

generally superior to retrospective studies. Data from studies independent of corporate sponsorship have particular value if the studies are well performed. The two systematic reviews on Ag-RDT accuracy consider this factor in their qu ality assessments (4,5).

2. **Reported performance**. Data demonstrating the performance of an Ag-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 obtaining many false-positive results. Most tests are achieving very high specificity, independent of the presence or absence of symptoms (4); however, as per the Annex, very low prevalence will still result in low positive predictive value. In that context, confirmatory testing needs to be considered based on level of suspicion/clinical history and the transmission scenario.

3. **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% in the target population, compared to NAAT. A useful assessment is the sensitivity of the test in patients with a rRT-PCR cycle threshold (Ct) below a specific value (e.g. 25-30), 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 several studies). It is important to note, however, that Ct values at a given input concentration of target RNA vary between rRT-PCR assays are not strictly quantitative. Recently, some investigators have been reporting Ag-RDT sensitivity based on detection of samples with a Ct cut-off associated with culturable virus or probability of culturing virus, a surrogate for sensitivity for infectiousness (57). It would be incorrect to assume that all samples above the Ct cut-off missed by most Ag-RDTs and detected by NAAT are non-infectious and therefore not clinically relevant. Although viral load is unquestionably a crucial factor in determining infectiousness, viral culture is not a very sensitive method itself and other factors are very likely to play a role in transmission, including symptoms like coughing and sneezing or behaviour (singing, talking, wearing masks) and the presence of neutralizing antibodies (63). Well-performing Ag-RDTs will likely detect the majority of infectious cases but establishing a cut-off for infectiousness is not currently scientifically feasible.

4. **Manufacturing quality and regulatory status**. Ag-RDTs, like 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.

5. **Procurement considerations.** The number of SARS-CoV-2 antigen detecting tests, both RDTs and those requiring immunoanalysers has massively expanded over the past year, with many new companies entering the market. The WHO document Procurement Considerations for COVID-19 Diagnostics provides practical advice for selecting and ordering diagnostic supplies, including Ag-RDTs. 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. Recent studies suggest close attention must be paid to temperature control. Specifically, ten-fold reductions in test sensitivity of many Ag-RDTs were reported after brief (10 minutes) exposure to elevated temperatures (37°C), and 30% of the products exposed to 2-4°C for 30 minutes had reduced specificity (64). In another study at a drive-through testing centre, the sensitivity and specificity of the Ag-RDT were 66.7% and 95.2% respectively in tests run at low temperature (8-14°C) on 30 specimens from individuals with symptoms ≤ 7 days. When testing was performed at > 15°C sensitivity was 93.7% and specificity 100% (65). Reductions in both Ag-RDT sensitivity and specificity have been reported in uncontrolled tropical settings (66).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. Currently, most commercial Ag-RDTs have a 12-month shelf-life and support transport and storage conditions only up to 30°C. This means that a cool chain and regular resupply mechanisms will be required for shipping, transport and storage in many countries and will 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; and trade-offs will need to be carefully considered. Krüger et al. developed an ease-of-use assessment form for SARS-CoV-2 Ag-RDTs (29).

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. As noted earlier, 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, but if confirmatory testing or serial testing is done this will increase overall costs of the testing strategy. 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. For Ag-RDTs purchased through the COVID-19 Diagnostic Consortium, there have been significant price reductions (30-40%) over the past 2 months (67).

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

Implementation considerations

The "who, what, when, where and how" of SARS-CoV-2 Ag-RDT usage alongside other testing modalities should be integrated into the national testing strategy.

For initial introduction of Ag-RDTs as part of testing programs, countries should ideally consider selecting some settings where NAAT confirmatory testing is available so that staff can gain confidence in the assays, confirm performance of the selected RDTs and troubleshoot any implementation issues encountered. Wherever NAAT will be used for confirmatory testing in individuals tested with 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. Complete recommendations for implementation are available in the WHO document SARS-CoV-2 antigen-detecting rapid diagnostic tests: an implementation guide. The following highlights – and findings since this publication appeared – are of particular note.

1. Ag-RDTs are easier to perform than NAAT but still **demand that manufacturer-recommended procedures be strictly followed.** All test operators must have training in sample collection, relevant biosafety and waste management, performance of the test and interpretation **and** reporting of results. Quality control measures also need to be put in place.

   Use of instrumented detection systems demands additional training in and sufficient infrastructure such as a reliable source of electricity.

   a. **Sample collection** is one of the most critical factors affecting performance of any diagnostic test, including Ag-RDTs. Inadequate or improper sample collection can result in false negative results. Instructions for use should be carefully followed, and any staff collecting samples should be trained in the methodology.

   b. Each Ag-RDT has specific **sample processing** requirements. Test-specific instructions should be followed precisely, and no alternative reagents used (e.g., water or other liquid instead of dilution/mixing buffer) or reagents exchanged between different brands of tests.

   c. **Biosafety** requirements for operators must be in place. Personal protective equipment, including a medical mask, gloves, eye protection and gown and good ventilation are essential (68). Although, some extraction buffers in the Ag-RDT kit will inactivate SARS-CoV-2 after several minutes of contact with the sample, it is recommended that all waste associated with performing the test be considered biohazardous, unless national authorities specifically instruct otherwise.

   d. Quality control measures

   Ag-RDTs include built-in procedural controls that verify that the sample has migrated to the intended location. Test manufacturers or third parties may also provide positive control materials with the test kits or sell them separately to verify that the test is accurate. The frequency of testing with controls should consider the manufacturer's instructions and needs to be established by the COVID-19 laboratory and the testing sites under its supervision. External quality assurance schemes are also emerging.

2. **Post-market surveillance**, with regulatory oversight, is critical to discover defects in products or accessories that negatively affect performance and potentially new variants that may compromise performance. The health system should ensure there is monitoring and evaluation of SARS-CoV-2 diagnostic testing activities and clear mechanisms for reporting problems.

3. **Variants.** Mutations in the viral genome may affect detectability by Ag-RDTs, either because of changes in the configuration of the target protein or in the abundance of the target virion. In the first case, for example, there are reports of mutations of the nucleoprotein gene that result in false-negative Ag-RDT results despite high viral loads confirmed by NAAT. These strains were found to have T205I and D399N mutations (69) or A376T and M241I mutations in an immunodominant epitope of the nucleoprotein (position 229-374) (70). Such samples with mismatched Ag-RDT and NAAT results should be prioritized for sequencing. WHO is monitoring for reports of diagnostic escape mutants and tracking their frequency in sequencing databanks. Thus far, there have not been any reports of reduced performance of Ag-RDTs in detecting any of the currently recognized variants of concern (71). It is worth noting that a preprint of research performed in individuals infected with the Delta variant reported a shorter incubation period and increased viral load (up to 1000 times higher) in respiratory samples compared to cases detected in the initial Wuhan outbreak in 2020 (72). Additional data is required to understand whether this is a fixed feature of Delta variants and whether this will impact Ag-RDT performance.

Future updates

WHO is working closely with groups evaluating the performance and operational characteristics of commercialized SARS-CoV-2 Ag-RDTs and various testing strategies to systematically compile the evidence as it emerges and to coordinate updates.

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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 guidnce. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance document will expire 2 years after the date of publication.

Annex

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. 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 Table 1, below). 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.

Table 1: 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 0,1, 0,5, 5, 10, 20, 30% and based on recommended performance criteria: sensitivity of 70, 80%, 90% and specificity of 97%, 98% and 99%.

Example target populations[a]	**Prevalence (%)**	**Sensitivity**	**Specificity**	**NPV**	**PPV**	**TP**	**FP**	**TN**	**FN**	**No. with disease**	**No. positive tests**	**Total**
Asymptomatic, no known exposure: travellers at points of entry, students; general population	0,1	50	97	99,9	1,6	50	2997	96903	50	100	3047	100000
		50	98	99,9	2,4	50	1998	97902	50	100	2048	100000
		50	99	99,9	4,8	50	999	98901	50	100	1049	100000
		70	97	99,97	2,3	70	2997	96903	30	100	3067	100000
		70	98	99,97	3,4	70	1998	97902	30	100	2068	100000
		70	99	99,97	6,5	70	999	98901	30	100	1069	100000
		80	97	99.,98	2,6	80	2997	96903	20	100	3077	100000
		80	98	99,98	3,8	80	1998	97902	20	100	2078	100000
		80	99	99,98	7,4	80	999	98901	20	100	1079	100000
		90	97	99,99	2,9	90	2997	96903	10	100	3087	100000
		90	98	99,99	4,3	90	1998	97902	10	100	2088	100000
		90	99	99,99	8,3	90	999	98901	10	100	1089	100000

Asymptomatic, no known exposure: travellers at points of entry, students	0,5	50	97	99,7	7,7	250	2985	96515	250	500	3235	100000
		50	98	99,7	11,2	250	1990	97510	250	500	2240	100000
		50	99	99,7	20,1	250	995	98505	250	500	1245	100000
		70	97	99,8	10,5	350	2985	96515	150	500	3335	100000
		70	98	99,8	15,0	350	1990	97510	150	500	2340	100000
		70	99	99,8	26,0	350	995	98505	150	500	1345	100000
		80	97	99,9	11,8	400	2985	96515	100	500	3385	100000
		80	98	99,9	16,3	400	1990	97510	100	500	2390	100000
		80	99	99,9	28,7	400	995	98505	100	500	1395	100000
		90	97	99,9	13,1	450	2985	96515	50	500	3435	100000
		90	98	99,9	18,4	450	1990	97510	50	500	2440	100000
		90	99	99,9	31,1	450	995	98505	50	500	1445	100000
	1	50	97	99,5	14,4	500	2970	96030	500	1000	3470	100000
		50	98	99,5	20,2	500	1980	97020	500	1000	2480	100000
		50	99	99,5	33,6	500	990	98010	500	1000	1490	100000
		70	97	99,7	19,1	700	2970	96030	300	1000	3670	100000
		70	98	99,7	26,1	700	1980	97020	300	1000	2680	100000
		70	99	99,7	41,2	700	990	98010	300	1000	1690	100000
		80	97	99,8	21,2	800	2970	96030	200	1000	3770	100000
		80	98	99,8	28,8	800	1980	97020	200	1000	2780	100000
		80	99	99,8	44,7	800	990	98010	200	1000	1790	100000
		90	97	99,9	23,3	900	2970	96030	100	1000	3870	100000
		90	98	99,9	31,3	900	1980	97020	100	1000	2880	100000
		90	99	99,9	47,6	900	990	98010	100	1000	1890	100000
Symptomatic general population; contacts of index case	5	50	97	97,4	46,7	2500	2850	92150	2500	5000	5350	100000
		50	98	97,4	56,8	2500	1900	93100	2500	5000	4400	100000
		50	99	97,4	72,5	2500	950	94050	2500	5000	3450	100000

		70	97	98,4	55,1	3500	2850	92150	1500	5000	6350	100000
		70	98	98,4	64,8	3500	1900	93100	1500	5000	5400	100000
		70	99	98,4	78,7	3500	950	94050	1500	5000	4450	100000
		80	97	98,9	58,4	4000	2850	92150	1000	5000	6850	100000
		80	98	98,9	67,8	4000	1900	93100	1000	5000	5900	100000
		80	99	98,9	80,8	4000	950	94050	1000	5000	4950	100000
		90	97	99,5	61,2	4500	2850	92150	500	5000	7350	100000
		90	98	99,5	70,3	4500	1900	93100	500	5000	6400	100000
		90	99	99,5	82,6	4500	950	94050	500	5000	5450	100000
Community transmission: Symptomatic patients presenting to health care facilities; contacts of index cases; institutions & closed communities with confirmed outbreaks	10	50	97	94,5	64,9	5000	2700	87300	5000	10000	7700	100000
		50	98	94,5	73,5	5000	1800	88200	5000	10000	6800	100000
		50	99	94,5	84,7	5000	900	89100	5000	10000	5900	100000
		70	97	96,7	72,2	7000	2700	87300	3000	10000	9700	100000
		70	98	96,7	79,5	7000	1800	88200	3000	10000	8800	100000
		70	99	96,7	88,6	7000	900	89100	3000	10000	7900	100000
		80	97	97,8	74,8	8000	2700	87300	2000	10000	10700	100000
		80	98	97,8	81,6	8000	1800	88200	2000	10000	9800	100000
		80	99	97,8	89,9	8000	900	89100	2000	10000	8900	100000
		90	97	98,9	76,9	9000	2700	87300	1000	10000	11700	100000
		90	98	98,9	83,3	9000	1800	88200	1000	10000	10800	100000
		90	99	98,9	90,9	9000	900	89100	1000	10000	9900	100000
Symptomatic at referral centre; Symptomatic or screening of health workers; care homes	20	50	97	88,6	80,6	10000	2400	77600	10000	20000	12400	100000
		50	98	88,7	86,2	10000	1600	78400	10000	20000	11600	100000
		50	99	88,8	92,6	10000	800	79200	10000	20000	10800	100000
		70	97	92,8	85,4	14000	2400	77600	6000	20000	16400	100000
		70	98	92,9	89,7	14000	1600	78400	6000	20000	15600	100000
		70	99	93,0	94,6	14000	800	79200	6000	20000	14800	100000

		80	97	95,1	87,0	16000	2400	77600	4000	20000	18400	100000
		80	98	95,1	90,1	16000	1600	78400	4000	20000	17600	100000
		80	99	95,1	95,2	16000	800	79200	4000	20000	16800	100000
		90	97	97,5	88,2	18000	2400	77600	2000	20000	20400	100000
		90	98	97,5	91,8	18000	1600	78400	2000	20000	19600	100000
		90	99	97,5	95,7	18000	800	79200	2000	20000	18800	100000
Symptomatic health worker/cleaners; care home residents	30	50	97	81,9	87,7	15000	2100	67900	15000	30000	17100	100000
		50	98	82,1	91,5	15000	1400	68600	15000	30000	16400	100000
		50	99	82,2	95,5	15000	700	69300	15000	30000	15700	100000
		70	97	88,3	90,9	21000	2100	67900	9000	30000	23100	100000
		70	98	88,4	93,8	21000	1400	68600	9000	30000	22400	100000
		70	99	88,5	96,8	21000	700	69300	9000	30000	21700	100000
		80	97	91,9	92,0	24000	2100	67900	6000	30000	26100	100000
		80	98	92,0	94,5	24000	1400	68600	6000	30000	25400	100000
		80	99	92,0	97,2	24000	700	69300	6000	30000	24700	100000
		90	97	95,8	92,8	27000	2100	67900	3000	30000	29100	100000
		90	98	95,8	95,1	27000	1400	68600	3000	30000	28400	100000
		90	99	95,9	97,5	27000	700	69300	3000	30000	27700	100000

a- prevalence estimates do not consider impact of vaccination in these example populations

WHO reference number: WHO/2019-nCoV/Antigen_Detection/2021.1