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

Interim guidance

6 October 2021

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

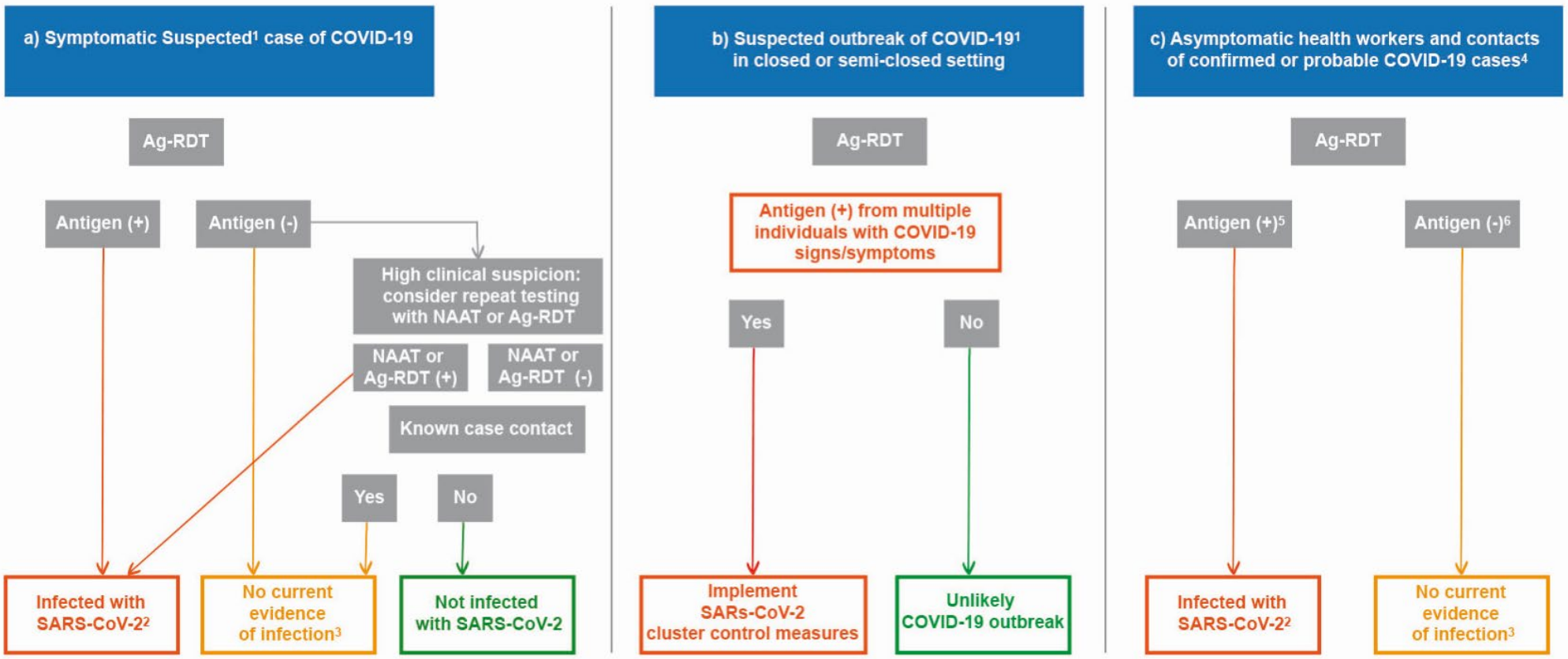
Because of their ease of use and rapid turnaround time, Ag-RDTs are a useful tool to quickly identify a cluster or outbreak and support the investigation and implementation of public health interventions to control transmission. The finding of positive Ag-RDT results from multiple individuals is highly 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%)

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