# **SARS-CoV-2 Antigen Rapid Diagnostic Test –**

# **Frequently Asked Questions**

## **Principles**

* **What is an RDT?**

RDT stands for rapid diagnostic test. RDTs are easy-to-perform tests that may be used outside of laboratory settings and typically give results in 15–30 minutes. Lateral flow tests are the most common type of RDT (eg. malaria tests).

* **What is an antigen?**

An antigen is a type of protein expressed by a virus, bacteria or parasite. It is recognized by the body’s immune system as something foreign. This triggers antibody production that specifically recognizes and neutralizes that antigen.

* **How does an antigen test work?**

An antigen test looks for antigens specific to a pathogen within a person's body to see whether they are infected with the target pathogen. For example, antigen tests for SARS-CoV-2 target detection of the nucleocapsid.

* **How do antigen tests differ from antibody tests?**

A positive antigen test reflects active infection, whereas a positive antibody test most likely reflects recent or past infection.

* **What is the difference between antigen testing and PCR?**

PCR detects the SARS-CoV-2 viral genetic material (RNA), whereas antigen tests detect SARS-CoV-2 specific antigens/proteins in a person's body. Both tests are used to detect active infection with SARS-CoV-2.

* **What do the markings on the RDT mean?**

In the test window (rectangular hole), a line near ‘C’ (for control) and a line near ‘T’ (for test) means that the test is positive for SARS-CoV-2. A line near ‘C’ and no line near ‘T’ means that SARS-CoV-2 was not detected. The line near ‘C’ is the control line; the presence of the control line tells you that the test was performed correctly. If a control line does not appear, it means that the test is invalid, even if a line appears near ‘T’. In this case, the patient must be re-tested using a new RDT. It is important to note that all results obtained from testing should be considered in conjunction with clinical history and other applicable data.

* **What are the sensitivity and specificity of SARS-CoV-2 Antigen RDTs?**

Reports on the performance of SARS-CoV-2 Antigen RDTs are increasingly being published. A systematic review of several reports can be found here: <https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013705/full> and FIND has also published reports here: <https://www.finddx.org/covid-19/sarscov2-eval-antigen/>.

Generally, reported test sensitivity is variable (0–94%), but is consistently better in the first five days following the onset of symptoms, when patients have the highest viral loads. WHO recommends the use of antigen RDTs that have a minimum of 80% sensitivity and 97% specificity based on good-quality, independent evaluations against an approved NAAT molecular assay. Some reports have demonstrated that these requirements can be met or even exceeded in some populations. Many factors will affect test performance, such as the characteristics of the patient population (e.g., number of days since onset of symptoms/viral load), quality of specimen collection and quality of test manufacture.

* **What is the underlying technology for the test?**

The underlying technology for the test is lateral flow immuno-chromatography (for further explanation see WHO’s *Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Interim guidance – 11 September 2020*:

 <https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays>).

* **What are the limitations of this test?**
* The test procedure, precautions and interpretation of test results must be followed strictly. Failure to accurately follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
* The test should be used for the detection of SARS-CoV-2 antigen in human nasopharyngeal swab specimens.
* Neither the quantitative value nor the rate of SARS-CoV-2 antigen concentration can be determined by this qualitative test.
* A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor-quality specimen is obtained.
* For more accurate assessment of immune status, additional follow-up testing using other laboratory methods is recommended.
* The test result must always be evaluated along with other data available to the physician.
* Positive test results do not rule out coinfections with other pathogens.
* Negative test results are not intended to rule out another coronavirus infection, only SARS-CoV-2.
* **How do variants affect the performance of this test?**

The SARS-CoV-2 virus has mutated over time, resulting in genetic variation in the population of circulating viral strains over the course of the COVID-19 pandemic. One consideration of how the mutations in these strains will impact antigen test performance is dependent upon whether the test detects nucleocapsid (N) or spike (S) protein. Healthcare professionals and laboratory personnel should be aware that false negative results may occur with \*\*any\*\* molecular test for the detection of SARS-CoV-2, particularly if a mutation occurs in the part of the virus' genome assessed by that test. Tests that have received WHO Emergency Use Listing Procedure (EUL) have not shown a decrease in their ability to detect SARS-CoV-2 virus variants.

## **Self-testing**

* **What is the benefit of self-testing?**
	+ COVID-19 self-testing is feasible, and self-testers can reliably and accurately perform SARS-CoV-2 Ag-RDTs, as compared to trained testers.
	+ Offering COVID-19 self-testing is acceptable and has the potential to achieve good uptake.
	+ Offering COVID-19 self-testing has the potential to enable timely diagnosis and prompt risk-based decisions and post-test actions, particularly in hard-to-reach communities.
	+ Offering COVID-19 self-testing has the potential to enable additional individual and social benefits including enabling individuals to make quicker post-test actions, including decisions that may affect their health and the health of their families and communities.
* **Can all Ag-RDTs be used for self-testing?**

Ag-RDTs that have been authorized for self-testing can be used. Typically, these tests are individually packaged with Instructions for Use (IFU) for each test. Professional use tests that have not been authorized for self-testing that have been re-packaged for self-testing should not be used.

* **How should the results of a self-test be used?**

For diagnostic purposes, an individual with a positive self-test result can be considered a probable case of SARS-CoV-2 infection and should take post-test actions, including infection control measures, according to current national guidelines. A negative self-test result is consistent with absence of current evidence of infection, but individuals should be made aware of the possibility of false-negative results. Individuals self-testing negative–especially those with persistent or progressing symptoms–should consider re-testing, e.g. 24 to 48 hours later and/or should seek testing for other diagnoses, including for other respiratory infections. Re-testing can be performed through a self-testing or through professional testing.

## **Specimen requirements**

* **What samples can I test using antigen tests?**

Nasopharyngeal swabs are the often-preferred sample for testing using SARS-CoV-2 Antigen RDTs. As new tests become available, other sample types are being used, such as nasal swabs. Always refer to the kit’s IFU which will specify the type of samples to use.

* **What samples are commonly used for self-testing?**

Nasal swabs are the often-preferred sample for self-testing using SARS-CoV-2 Antigen RDTs. As new tests become available, other sample types are being used, such as throat swabs or saliva. Always refer to the kit’s IFU which will specify the type of samples to use.

* **How long can samples be stored after collection before testing?**

Follow the manufacturer’s instructions for use. Some test samples should be tested within four hours of collection when stored at ambient temperature.

* **How should samples be stored while awaiting testing or during shipment?**

Follow the manufacturer’s instructions for use. In some cases, samples can be stored at 4–30°C prior to testing. If the ambient temperature is over 30°C, samples should be stored in a refrigerator or cool box during transportation and prior to testing.

* **The sample is bloody, can I still use it for testing?**

Yes. Samples that contain traces of blood can still be used for testing with an antigen test.

* **Can antigen RDTs be used to test blood samples?**

No. Antigen RDTs are currently approved for testing nasopharyngeal aspirates.

* **Can antigen RDTs be used to test saliva?**

No. Antigen RDTs are currently approved for testing nasopharyngeal aspirates. Evaluations are ongoing to assess performance with other specimen types.

## **Safety**

* **What safety requirements are needed for collecting samples?**

Personal protective equipment (PPE), including gloves, eye protection, medical mask and gown, must be worn while collecting samples from persons being investigated for COVID-19 infection (see WHO’s *Personal protective equipment for COVID-19:*

<https://www.who.int/medical_devices/priority/COVID_19_PPE/en/>).

* **What safety requirements are needed for performing antigen RDTs?**

Personal protective equipment (PPE), including gloves, eye protection, medical mask and gown, must be worn while performing RDTs.

* **What special precautions should I follow when testing?**

Adhere strictly to the manufacturer’s instructions for use and standard operating procedures (SOPs).

* **How should I dispose of waste (e.g., used tests, sample containers, etc.)?**

Unless indicated otherwise in the IFU, all waste generated from the testing of specimens from suspected or confirmed COVID-19 cases should be classified as biohazardous waste and should be handled according to applicable local guidelines.

Note that some antigen RDT kits’ extraction buffers, when used according to the IFU, will inactivate SARS-CoV-2 virus when the sample is added to the buffer in the extraction tube. Indicated waste management procedures may be found in the IFU.

## **Pre-testing**

* **How should I store the kit?**

Follow the manufacturer’s instructions, typically found on the side of the box for test kits. Typically, the kit should be stored at 2–30°C / 36–86°F out of direct sunlight.

* **What is the shelf life of the kit?**

Kit materials are stable until the expiration date printed on the outer box. Typically, shelf life is 12 months from the date of manufacture.

## **Testing**

* **How much sample should be used?**

See the manufacturer’s instructions for use.

* **What if I added too much sample or too little sample?**

Adding too much or too little sample can prevent the device from working correctly, thereby generating an invalid result or a result that might be difficult to read.

* **What factors can potentially affect the quality of the test?**
* The quality of the sample
* Using expired test kits
* Exceeding the recommended storage conditions for the kits
* Poor packaging
* Not following the instructions for use.
* **How long should the test be incubated before reading the result?**

Follow the manufacturer’s instructions for use. Typically, results are read in 10–30 minutes. Do not read after the maximum time limit set by the manufacturer, as test results in such situations are unreliable and may give false positive or negative results. If there is any doubt, testing should be repeated.

## **Interpreting results**

* **What does an invalid or unsuccessful antigen RDT result mean?**

Occasionally, a person will have an invalid or unsatisfactory test result, which may be the result of excessive mucus in the sample, which interferes with the test, or other issues with specimen collection. This person should have a repeat swab collected and sent for SARS-CoV-2 testing.

* **Can a patient with a negative antigen RDT result still have COVID-19 infection?**

Yes. Persons infected with COVID-19 may have a negative test result. Current data suggest that 15–20% of patients may be missed with antigen tests.

* **Should I re-test patients with a negative antigen RDT result using a second RDT?**

It is preferable to re-test antigen RDT-negative patients with a more sensitive test such as RT-PCR. However, if molecular-based testing is not available, then repeat testing with a second antigen RDT in the subsequent 48 hours can be considered, especially if symptoms persist or progress.

* **Should I confirm a positive antigen RDT result with PCR?**

If RDTs are used to diagnose symptomatic patients or asymptomatic contacts of cases in areas with widespread community transmission, the positive antigen RDT result is sufficient to confirm a case and confirmatory testing is not needed. However, if antigen RDTs are used in low prevalence settings, positive results may be more likely to be false positives than true positives, and confirmatory testing of RDT positives is strongly advised.

* **No control band is visible on the test after incubation for the required time. What should I do?**

Take a new sample and repeat the test.

* **What can cause a false negative test result?**

False negative results (a negative test result from a patient with COVID-19 infection) can occur when the amount of viral antigen in the sample is below the limit of detection of the test. This may happen because of poor sample collection methods or sampling when viral loads are low, such as in very early disease or later in the illness, e.g., more than seven days post onset of symptoms. Tests work best when persons are tested within the first five to seven days after the onset of symptoms.

* **The control (C) and the test (T) band are both visible. What does this mean?**

When both the control and test bands are visible, it means the test is positive.

* **The control (C) band is visible, but the test (T) band is not visible. What does this mean?**

When the control band is visible, but the test band is not visible, it means the test is negative.

## **Where can I find more information on SARS-CoV-2 diagnostics?**

* World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Interim guidance – 11 September 2020: <https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays>
* World Health Organization. Country & technical guidance – Coronavirus disease (COVID-19): <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance>
* World Health Organization. Diagnostic testing for SARS-CoV-2. Interim guidance – 11 September 2020: <https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2>
* World Health Organization. Coronavirus disease (COVID-19) pandemic – Emergency Use Listing Procedure (EUL) open for in vitro diagnostics: <https://www.who.int/diagnostics_laboratory/EUL/en/>
* World Health Organization. Use of SARS-CoV-2 antigen-detection rapid diagnostic tests for COVID-19 self-testing. Interim guidance- 9 March 2022. Geneva. World Health Organization. <https://www.who.int/publications/i/item/WHO-2019-nCoV-Ag-RDTs-Self_testing-2022.1>
* World Health Organization. Post-market surveillance for in vitro diagnostics (IVDs): <https://www.who.int/diagnostics_laboratory/postmarket/en/>
* World Health Organization. Personal protective equipment for COVID-19:
* <https://www.who.int/medical_devices/priority/COVID_19_PPE/en/>
* Centers for Disease Control and Prevention. Coronavirus disease 2019 (COVID-19) – Guidance documents: <https://www.cdc.gov/coronavirus/2019-ncov/communication/guidance-list.html?Sort=Date%3A%3Adesc>
* Africa Center for Disease Control and Prevention. COVID-19 guidance on use of personal protective equipment for different clinical settings and activities, May 2020:

<https://africacdc.org/download/covid-19-guidance-on-use-of-personal-protective-equipment-for-different-clinical-settings-and-activities/>

* US Food and Drug Administration. SARS-CoV-2 Viral Mutations: Impact on COVID-19 Tests. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests.

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