An Advocates' Introduction to SARS-CoV-2 Rapid Diagnostic Testing

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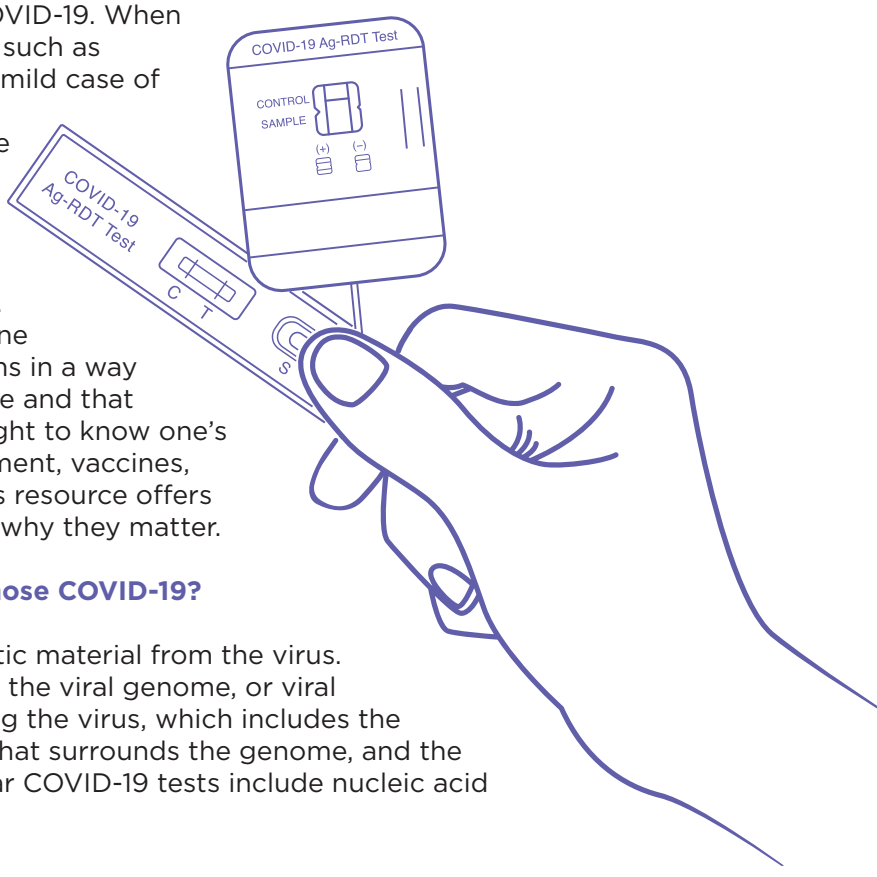
May 2022

All people have the right to know their COVID-19 status and to be able to use that information to protect themselves, their families, and their communities. Yet many people do not have this information. Since March 2020, only 21.5% of the COVID-19 tests administered worldwide have been used in low- and lower-middle-income countries, which make up more than half of the world's population.[1] In late 2021, the World Health Organization (WHO) estimated that six out of seven SARS-CoV-2 infections in people living in Africa were undetected.[2]

This resource is a tool for advocates who want information about tests for themselves and their communities and to inform advocacy campaigns to secure access to testing. It describes the basics of SARS-CoV-2 diagnosis and focuses specifically on rapid antigen tests (Ag-RDTs). Ag-RDTs are especially accurate for diagnosis in symptomatic individuals who can then be linked to effective treatments. These tests are widely used in high-income countries for individual decision-making and risk management. They do not require cold chain for laboratory or transport, and are often more affordable than other testing options. The quick turnaround time of Ag-RDTs means that these tests are the key for connecting people to treatment for COVID-19. When started soon after testing positive, treatments such as nirmatrelvir/ritonavir (Paxlovid) can prevent a mild case of COVID-19 from progressing to severe disease and may quicken recovery time and reduce the amount of time a person is infectious.



It is important that all countries and national health programs secure access to a stable supply of affordable, quality-assured Ag-RDTs. Advocacy is urgently needed to demand routine access to these tests in test-and-treat programs in a way that is voluntary, confidential, and non-coercive and that recognizes the right to health, including the right to know one's SARS-CoV-2 status and obtain effective treatment, vaccines, and other protective measures as needed. This resource offers more information on what these tests are and why they matter.

What are the different types of tests to diagnose COVID-19?

1) **Molecular COVID-19 tests** detect genetic material from the virus. Genetic material (also known as genes, the viral genome, or viral RNA) carries the instructions for making the virus, which includes the genome, the protein cage or "capsid" that surrounds the genome, and the "envelope" that contains it all. Molecular COVID-19 tests include nucleic acid

amplification tests (NAATs), such as real-time reverse transcriptase polymerase chain reaction (rRT-PCR) tests.

2) **Antigen tests** (Ag-RDTs) detect viral proteins. Proteins are large, complex molecules that support the structure and direct most of the functions of living organisms. Viral structures like the capsid and the envelope contain many different proteins. (The SARS-CoV-2 protein that docks to human cells is called the Spike protein.) Antigen tests (Ag-RDTs) are also known as COVID-19 rapid tests and are the focus of this guide.

Also of note is a third type of test, called an **antibody test**, that detects immune responses to SARS-CoV-2. An antibody test can be used to determine whether a person has *ever* had SARS-CoV-2 or to measure the immune response to a vaccine. Because antibodies stay in the blood after the virus is gone, antibody tests cannot tell whether a person is currently infected. Therefore, antibody tests *should not* be used to diagnose active SARS-CoV-2 infection.

What are some key similarities and differences between the molecular and antigen tests?

SARS-CoV-2 is a respiratory virus, which means it can be found at the surfaces where we breathe: inside the nose, mouth, and throat. Both types of tests collect samples at or from these places:

- Saliva;
- Swabs from the inside of the nose (this can include long oropharyngeal swabs that reach deep inside the nostril and swabs inserted at more shallow depths);
- Swabs from the tongue, cheeks, or tonsils/back of the throat.

Table 1: Molecular and Antigen Tests: The Basics

	Highly sensitive	Requires cold chain for transportation and storage	Requires a laboratory for analysis	Returns results quickly (<1 hour)	Can be self-administered
Molecular tests (NAATs, PCR)	✓	✓ (most of the time)	✓		
Antigen tests (Ag-RDTs)				✓	✓

Molecular tests use chemicals and detection technology to locate and make many copies of — or amplify — any viral RNA present in a sample. Molecular tests can deliver an accurate positive result even if there is a tiny amount of viral RNA from a sample. The tests are done in laboratory settings, and it can take hours or days for the results to be available. The turnaround time depends on the test used, how many samples the lab is able to process in a day, and how far the sample has to travel from where it was collected to the laboratory for processing, among other factors. Some molecular tests can detect SARS-CoV-2 in as little as 30 minutes, but these are not yet widely available.

Antigen tests look for viral proteins and do not use any kind of amplification. The tests typically come as a kit with several components: a swab, a test strip, and a small vial of liquid chemical solution (Figure 1). The test strip usually has a viewing window where the result is read. This window contains two lines: a Control line (which is always visible) and a Test line (which becomes visible if the test is positive). The sample is collected with the swab and then mixed with the chemical solution in the vial that is then applied to the test strip. The test strip contains molecules that attach to viral proteins, if they are present. When this happens, the test strip shows a sign near the Test line area — usually a colored line — indicating a positive result (Figure 2).

Figure 1: Example of an Ag-RDT Test Kit

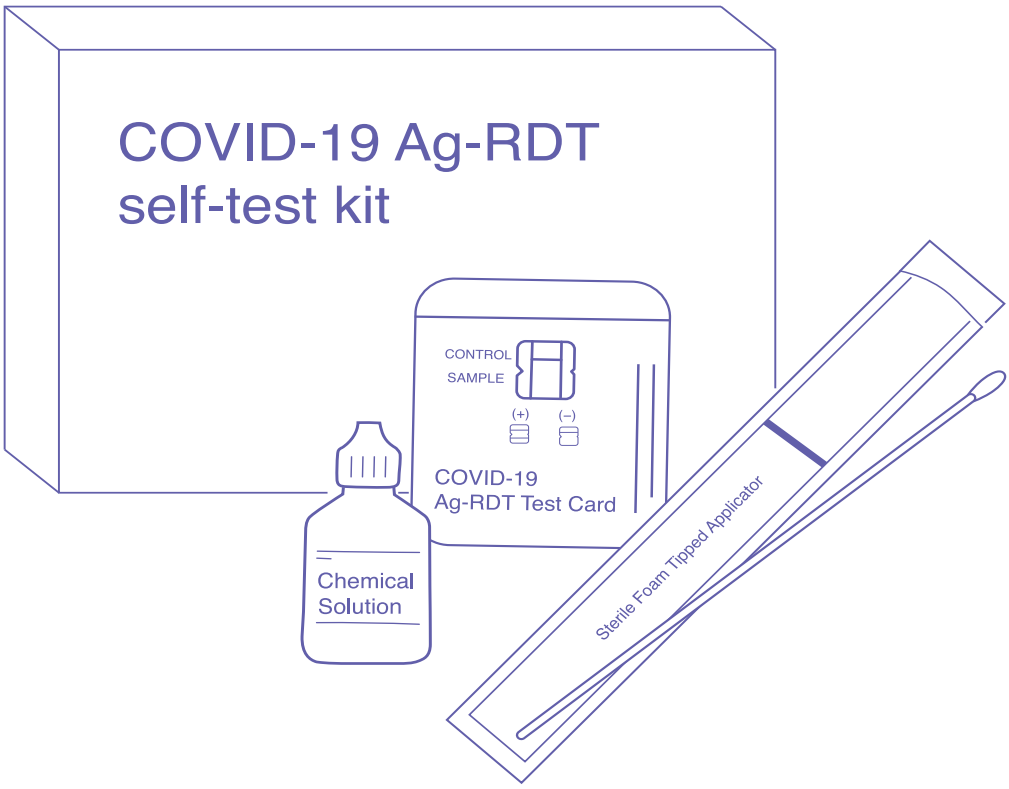
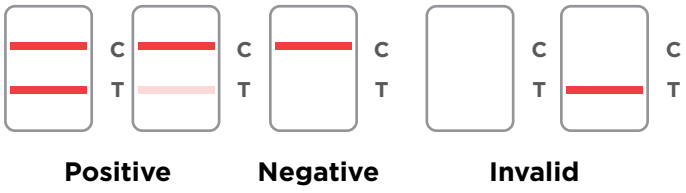


Figure 2: Reading Ag-RDT Test Results



C = control line (should always be visible)

T = test line (becomes visible when test is positive).

- A faint, light test line is positive.
- A bold, dark test line is positive.

Antigen tests look and work much like at-home pregnancy tests and HIV self-tests: the person collects the specimen themselves, performs the test, and interprets the result. People can choose when and where to test. Antigen tests provide results quickly, often within fifteen minutes of the sample being placed on the test strip. The tests do not require laboratories for processing and they can be performed at the point of care (POC) where treatment is available — for example a clinic, a pharmacy, or in the presence of a community health worker. This means that a person can start treatment immediately after the diagnosis. They can also be performed at home and used to guide a decision about seeking treatment, re-testing with another Ag-RDT 24–48 hours later, or seeking confirmatory molecular testing.

It's important to note that nirmatrelvir/ritonavir (Paxlovid) is only effective within the first five days after exposure or onset of symptoms, so moving quickly to test if you are feeling sick is key. Since Ag-RDTs can be self-administered, they are sometimes called self-tests, meaning someone can perform all the steps in the testing process themselves without the aid of a health care worker. A small percentage of antigen tests is analyzed in laboratories.

Table 2: Molecular and Antigen Tests for SARS-CoV-2: A Summary Overview*		
	Molecular tests (NAATS, PCR)	**Antigen tests (Ag-RDT)**
Intended use	Detects current infection	Detects current infection
What it measures	Viral ribonucleic acid (RNA)	Viral proteins (antigens)
Specimen types	Nasal, nasopharyngeal, oropharyngeal, sputum, saliva	Nasal, nasopharyngeal, saliva
Sensitivity	Varies test by test but generally high for laboratory-based tests and moderate to high for point-of-care–based tests	Varies depending on the course of infection, but generally moderate to high when people are symptomatic (at times of peak viral load)
Specificity	High	High
Can be used at the point of care (POC)	Mostly no — with some exceptions	Mostly yes — with some exceptions
Requires a cold chain for transportation and storage	Mostly yes	No
Turnaround time	Varies based on lab capacities, demand, sample transport time — most require a minimum of days; some can be done in hours	Ranges from 15 to 30 minutes
Cost per test**	US$10.00–19.00 per rRT-PCR test for automated use; US$4.70–10.00 for manual use	US$0.78–5.00 per test
Advantages	Most sensitive test method available Usually does not need to be repeated to confirm results	Short turnaround time (approximately 15 minutes) When performed at or near POC, allows for rapid identification of people with SARS-CoV-2, thus preventing further virus transmission in the community, workplace, or home Comparable performance to molecular tests in symptomatic persons and/or if culturable virus is present, when the person is presumed to be infectious
Disadvantages	Longer turnaround time for most molecular tests compared with Ag-RDTs Higher cost per test compared with Ag-RDTs Will return positive test results after risk of transmission has passed	May need confirmatory testing (but not when someone is symptomatic or has had known contact with someone with COVID-19) Less sensitive (more false negative results) compared with molecular tests, especially among asymptomatic people
Note: **Sensitivity** refers to the accuracy of the test in detecting the presence of SARS-CoV-2. More precisely, sensitivity is the proportion of people with a disease that the test correctly identifies as having the disease. **Specificity** refers to the accuracy of the test to rule out the presence of SARS-CoV-2. More precisely, specificity is the proportion of people without a disease that the test correctly identifies as not having the disease.		
*Table is adapted from the U.S. Centers for Disease Control and Prevention. *Guidance for antigen testing for SARS-CoV-2 for healthcare providers testing individuals in the community*. Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html#anchor_1631295369851. **The Global Fund maintains a COVID-19 diagnostics reference pricing document that is updated regularly. *Pooled procurement mechanisms reference pricing: COVID-19 diagnostics*. Available at: https://www.theglobalfund.org/en/covid-19/health-product-supply/diagnostics-procurement/.		

How are Ag-RDTs and other diagnostics used?

Tests can be used for many reasons:

- Diagnosing people;
- Screening people who want to gather, hold an event, or make decisions in a workplace, school, or other setting;
- Providing a population-level picture of how many people have or have had SARS-CoV-2.

Diagnosis, screening, and population-level tracking, also called epidemiological surveillance, are all important. A robust, comprehensive response to SARS-CoV-2 should include funding and programming for all of these activities.

Box 1: Interpreting Ag-RDT Test Results: What Do You Do with One Line — or Two?

Ag-RDTs are an affordable, accessible diagnostic. Follow these guiding principles for reading results to use them as an effective and reliable tool:

If you took an Ag-RDT and tested positive, you are likely to have active SARS-CoV-2 infection. This is particularly true if you are experiencing symptoms, have recently been exposed, and/or live in a community with high rates of SARS-CoV-2 transmission. The higher the prevalence (percentage of the population around you with the virus), the more likely it is that a positive test result is accurate. If you are someplace with very, very low prevalence, a positive result might be a false positive.

➔ ***You should***: **Seek treatment if you are symptomatic, are immunocompromised, or have other conditions that put you in a high-risk group for severe disease.** If you have symptoms, have had an exposure, and/or are in a high-prevalence setting, you should not be required to have a confirmatory molecular test prior to accessing treatment. If you are positive, have no symptoms or known exposure, and live in a low-prevalence area, you can consider re-testing with an Ag-RDT in 24–48 hours.

If you took an Ag-RDT and tested negative, you may (or may not) need to test again. If you are experiencing symptoms, have recently been exposed, and/or live in a community with high rates of SARS-CoV-2, a negative Ag-RDT may be a false negative.

➔ ***You should:*** **Seek a confirmatory molecular test right away if you're feeling sick.** If you are not able to get a confirmatory molecular test and are experiencing symptoms, re-test with an Ag-RDT in 24–48 hours. If you are not feeling symptomatic, live in a low-prevalence area, and haven't had a recent exposure, then there's a strong chance that the negative result is accurate.

Figure 3 *Test to Treat and Protect* (at the end of this guide) illustrates these guiding principles with a flow chart of actions a person can take based on Ag-RDT test results.

With both HIV and SARS-CoV-2, viral load is highest in the period immediately following infection; this is also when people are most likely to pass on the virus. Ag-RDTs are more likely to give a positive result when there is a substantial amount of virus in a person. This makes them a very useful tool for confirming infection in someone who is sick, and for identifying people — whether or not they are experiencing symptoms — who are likely to transmit. The WHO interim guidance on Ag-RDTs states that these tests are "effective for identifying people who are most infectious" and notes that a limitation of molecular tests is that they can return a positive result "well past the period of transmission and recovery." Molecular tests detect *any* amount of virus. In contexts where a negative test result is a prerequisite, like air travel, border crossings, or entry into workplaces, molecular tests can prolong quarantine periods past the point needed to avoid risk of transmission.

Ag-RDTs can also be used to screen people who want to gather for an event, as in a congregation or in a school, and by people who have had a known exposure to someone with SARS-CoV-2. In testing after exposure or for screening, it's important to remember that people do not have detectable virus right away. A negative Ag-RDT does not mean that a person isn't carrying SARS-CoV-2. They could be early in the infection period. The accuracy of Ag-RDTs increases with what is called "serial testing." A person with an initial negative result who is actually infected is likely to receive a positive result if they test 1–2 days later. The U.S. Centers of Disease Control and Prevention recommends testing five days after exposure. If you are experiencing symptoms, test sooner.

In high-income countries, while there are some gaps in access to and use of Ag-RDTs, for many people, using these tests as a decision-making tool is a part of daily life. Often, the results are not reported back to public health authorities — especially if they are negative. This would also be the case for Ag-RDTs that are used in low- and lower-middle-income countries, too. The right to know one's status should not be placed in competition with national priorities for tracking the virus and making public health decisions. Individual health and public health are both important.

Scaling up access to Ag-RDTs for self-testing and at clinics and other points of care is critical to successful test-and-treat programs.

A person who gets a positive test result after self-testing with an Ag-RDT has the option of isolating, masking, or distancing and has the right to access treatment. There are now highly effective treatments for SARS-CoV-2, including treatments for mild or moderate COVID-19 that can be taken outside of the hospital. For example, a treatment called nirmatrelvir/ritonavir (Paxlovid) is effective if started within three to five days of first experiencing COVID-19 symptoms. Scaling up access to Ag-RDTs for self-testing and at clinics and other points of care, like pharmacies, is critical to successful implementation of test-and-treat programs. In places where infections are surging, the most vulnerable groups — including older people, frontline health workers, people living with HIV, or people with tuberculosis (TB) — could receive Ag-RDTs and a course of medication in the event of infection.

Are all Ag-RDTs reliable?

High-quality Ag-RDTs are most accurate in the context of symptomatic infection; accuracy increases with serial testing. As discussed in Box 1 on page 5, accuracy is also affected by how much SARS-CoV-2 is circulating in your community or workplace. If you live or work someplace where the risk of SARS-CoV-2 is high, a positive result very likely reflects actual infection. If you live or work someplace with little or no SARS-CoV-2, the tests are better at predicting that you don't have SARS-CoV-2. In other words, a negative result very likely means that you don't have SARS-CoV-2. When there's high community or workplace transmission (i.e., a health facility), a negative result is more likely to be a false negative. This is especially true if you've had a known exposure or are experiencing symptoms. See Box 1 and Figure 3 for more on how to use tests most effectively.

All of the above is true for high-quality Ag-RDTs. As for all medicines, vaccines, and diagnostics, there is a regulatory review process for Ag-RDTs to ensure they meet quality standards. High-quality tests are tests that have received use authorizations and/or approval from stringent regulatory authorities; they do not give positive test results with drinking water or other fluids; they do not come with predetermined results; and they are all capable of distinguishing between SARS-CoV-2 and other viruses. Rumors and misinformation about test accuracy exist. Peer-based literacy work to share accurate information and understand and debunk myths is crucial, as is advocacy to secure access to high-quality tests.

If someone has been vaccinated or had SARS-CoV-2, do Ag-RDTs still work?

Yes. Ag-RDTs detect viral antigen, or protein, indicating active infection. The vaccines teach the body how to make immune responses and do not leave antigen in the body. If you have had SARS-CoV-2 and the symptoms of acute infection have passed, the virus may still be present in the body at low levels, but these will not be detected by an Ag-RDT.

Do SARS-CoV-2 tests diagnose "long COVID"?

While estimates vary, somewhere between ten and forty percent of people who acquire SARS-CoV-2 experience prolonged, chronic symptoms. These symptoms vary across individuals and over time in the same person; they are often different from the symptoms experienced during initial infection. Long COVID can be debilitating and is a crucial health concern. Vaccination may reduce the risk of long COVID; people living with HIV may be at higher risk of long COVID than those in the general population. Ag-RDTs and PCR tests do not diagnose this chronic condition, which emerges after the virus is cleared from the body.

Do Ag-RDTs detect Omicron (or other) variants?

Ag-RDTs do detect Omicron (BA.1, BA.2) in addition to other variants of SARS-CoV-2. There are mixed data on whether these tests are less accurate with Omicron than with other variants. A group of Canadian researchers analyzed and compared the findings of three papers on Ag-RDT detection of Omicron and four papers on Ag-RDT detection of Delta to better understand accuracy with different variants.[3] In this analysis, Ag-RDTs using nasal swabs were less reliable at detecting Omicron than they were for correct diagnosis of Delta variants; tests that combined oral (cheek, tongue, or back of the throat) and nasal samples had better accuracy. Another study of over 5,500 people in the United States found no difference in accuracy of nasal swab Ag-RDT detection of Omicron versus Delta.[4] The possibility of reduced accuracy is one reason why effective testing programs will include easy, affordable, and/or free Ag-RDTs as well as easy, affordable, or free molecular tests for confirmatory diagnosis. FIND maintains a dashboard of the impact of variants on the accuracy of diagnostic tests that has the most up-to-date information.[5]

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Take action! What must happen to expand access to Ag-RDTs in low- and lower-middle-income countries?

Many steps need to be taken to close the access gap for SARS-CoV-2 diagnostics. Here are some of the crucial actions.

Guidance:

In March 2022, the WHO issued interim guidance on the use of Ag-RDTs for self-testing.[6] This is a prerequisite for many low- and lower-middle-income countries to develop national guidelines and operational plans. (The WHO has yet to issue guidance on test-and-treat for SARS-CoV-2, which is also crucial.)

Health ministry SARS-CoV-2 taskforces must develop national Ag-RDT guidelines, including for self-testing, with robust civil society input and support as needed from multilateral and bilateral funders. This guideline development process should proceed in tandem with and be informed by planning for SARS-CoV-2 treatment roll out.

Regulatory Approval and Supply:

As of February 2022, the WHO had recommended three Ag-RDTs for Emergency Use Listing (EUL). These three manufacturers do not have capacity to supply enough tests to meet the testing targets adopted by the U.S. government (one test per 1,000 people per day[7]) and the WHO (10 tests per 10,000 people per week).[8,9] The Global Fund to Fight AIDS Tuberculosis and Malaria (Global Fund) lists a range of other Ag-RDTs that can be procured through its Emergency Response Mechanism.[10] As the Access to COVID-19 Tools (ACT) Accelerator civil society representatives have articulated:

It is imperative that the WHO "expedite emergency use listing and facilitate rapid regulatory approval at the national level." Funders, including the U.S. government, should take action now to secure and stockpile supplies of Ag-RDTs in anticipation of national-level guidelines and additional EULs for tests, particularly those that have already been authorized for use by a stringent regulatory authority, for example the European Medicines

Agency or the U.S. Food and Drug Administration. Ag-RDTs are simple to manufacture, yet capacity is concentrated in a few countries. Technology transfer and investments in geographically distributed manufacturing are critical.

Cost and affordability:

The current price for Ag RDTs is between US$0.78 and US$5.00 per test, according to the Global Fund reference list. However, the price must drop further, to an average of US$1.00 per test — a price reported in certain high-income countries, including the UK and Germany — or even lower.

Bulk procurement by stakeholders including USAID, the Global Fund, international agencies, and governments — and sustainable competition via expanded manufacturing and additional EULs by the WHO for existing manufacturers — should be pursued with the goal of achieving US$1.00 a test or less.

Community-led Introduction and Implementation:

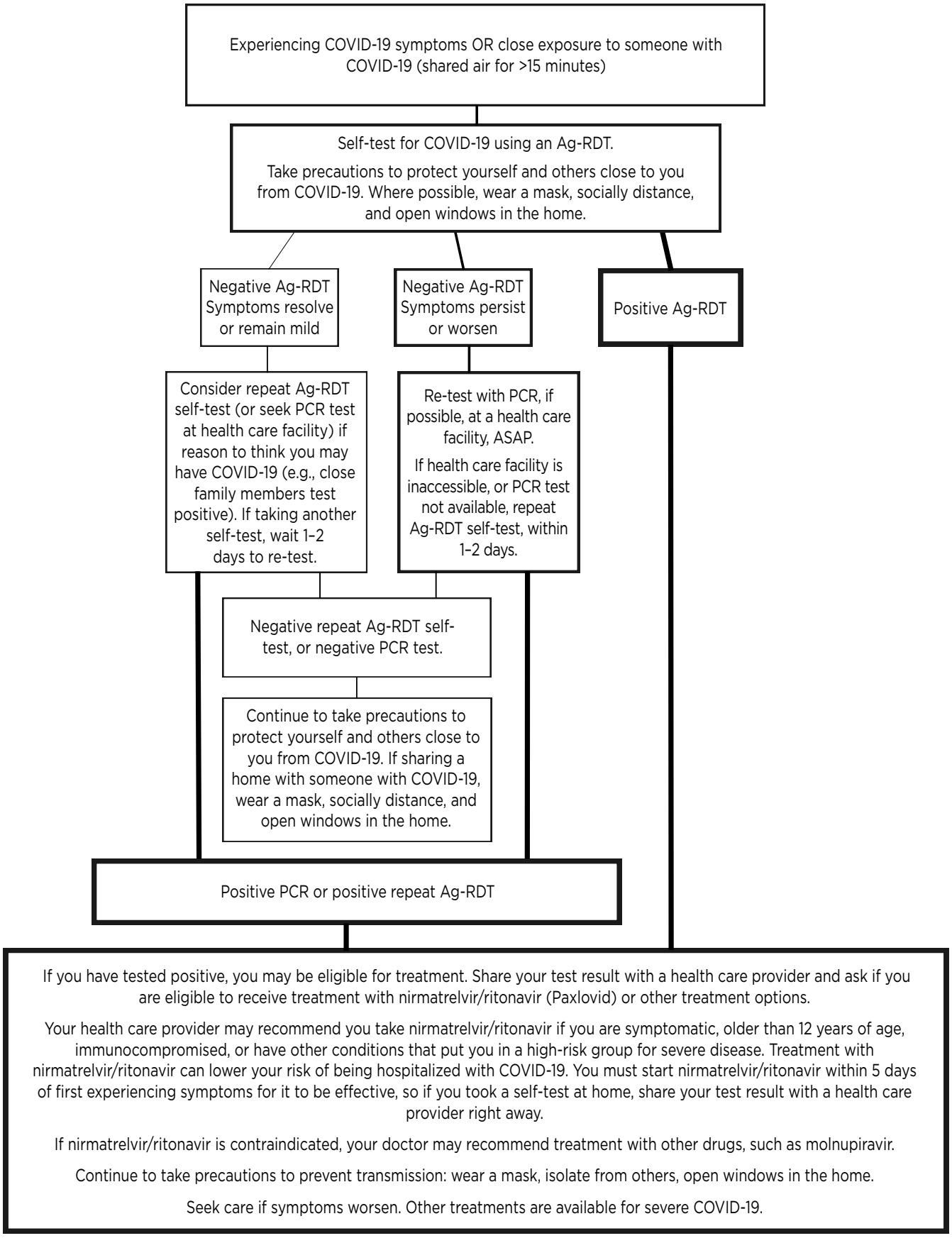
Uptake of testing depends on trust in the test, the person or site offering it, and the larger health system. People will seek tests if they are confident that treatment and care will be readily available, if they believe that the diagnosis will not bring shame, blame, or stigma, and when they have clear information about risks and benefits. These aren't new lessons, yet they still aren't routinely followed — even in HIV and TB programs.

People living with and at risk of HIV and TB also have a wealth of experience with novel service delivery models that should be used as part of effective test-and-treat programs. For example: rolling out Ag-RDTs with treatment kits to people living with HIV with their antiretroviral therapy refills during surge periods; putting these kits into the supplies given to community health workers; or deploying them as part of HIV pre-exposure prophylaxis (PrEP) refill and/or TB preventive treatment distribution programs. Moving testing and treatment closer to people most at risk increases uptake and reduces cost.

Investments in treatment and diagnostic literacy led by and for people at risk of SARS-CoV-2 is crucial to build trust and create service delivery models that work and expand uptake as Ag-RDTs and treatment become available.

People most impacted by pandemics must be the ones to set priorities and lead responses — otherwise the pandemics will not end.

Figure 3: Test to Treat and Protect: Self-Testing as an Entry Point for COVID-19 Treatment



Endnotes

[1] Estimate from March 2022. For up-to-date information visit the FIND testing tracker: https://www.finddx.org/covid-19/test-tracker/

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