

# Gold-standard RT-PCR detection of SARS-CoV-2 at rapid speed: a solution for asymptomatic screening

## Key messages

- Decentralized screening for SARS-CoV-2 infection is an essential tool in our recovery from the SARS-CoV-2 pandemic.
- Rapid tests detecting virus antigens and RNA are available but must be implemented with consideration for potential limitations in performance.
- The Thermo Fisher Scientific™ Accula™ SARS-CoV-2 Test is an ideal solution that addresses requirements for timely results with high sensitivity and specificity.

## Introduction

SARS-CoV-2 testing remains an essential tool as we seek to restore the health of communities globally. In ~40–45% of cases, infection with SARS-CoV-2 does not lead to symptomatic disease [1], and it is estimated that up to 50% of new infections originate from exposure to individuals without symptoms (asymptomatic) [2]. Screening tests are intended to identify infected individuals prior to development of symptoms or those infected asymptomatic individuals who may be contagious, so that measures can be taken to prevent those individuals from infecting others [3]. Thus, screening of asymptomatic persons is critical to reducing transmission and enabling a safe return to work, school, and community activities.

The gold standard for detection of SARS-CoV-2 is the reverse-transcription polymerase chain reaction (RT-PCR) due to its high analytical sensitivity and specificity. Standard RT-PCR relies on specialized materials and instruments, highly trained personnel, and transportation of specimens to a centralized laboratory. Samples are batched (i.e., many are run at the same time on a high-throughput instrument), and time-to-results can vary from several hours to several days.

For screening to be impactful, users must be able to receive results quickly after sampling. Requiring significant

quarantine time between sampling and test results is impractical for most school or workplace settings and community gatherings, and rapid turnaround time for results is essential in order to minimize exposure to infected persons and optimize contact tracing. The need for decentralized alternatives to lab-based testing necessitates a more scalable approach for screening, and rapid tests are being utilized to meet this need.

Rapid SARS-CoV-2 tests offer:

- **Ease of use**—no need for highly trained operators or specialized settings
- **Speed**—provide results in <1 hour
- **Cost**—less expensive than standard laboratory tests

It can be challenging to translate scientific information on SARS-CoV-2 tests into effective, widespread implementation in nonclinical settings. This paper intends to educate and dispel some common misconceptions regarding rapid tests—focusing on the science, regulatory guidance, and appropriate applications for screening asymptomatic populations.

## Test performance

There are two categories of rapid tests for SARS-CoV-2: nucleic acid amplification tests (NAATs) and antigen (Ag) tests. Rapid NAATs detect viral RNA (e.g., by RT-PCR or isothermal amplification), and rapid Ag tests are immunoassays that detect the presence of a specific viral antigen (protein). Rapid tests differ in performance characteristics, most significantly in analytical sensitivity.

The exponential amplification of nucleic acid targets by NAAT methods enables detection of very small amounts of SARS-CoV-2 RNA in a specimen. Rapid NAATs that employ isothermal amplification do not require the sophisticated thermal cycling involved in RT-PCR but are less sensitive than both rapid RT-PCR and standard lab-based RT-PCR [4]. Ag tests do not amplify their protein targets, so they are generally less sensitive than most NAATs [5].

### NAATs

Tests within each category (NAAT or Ag test) do not have equivalent performance and should be evaluated on an individual basis. The FDA established a Reference Panel for NAAT SARS-CoV-2 tests, enabling direct comparison of limit of detection (LOD) across Emergency Use Authorization (EUA) tests, utilizing standardized material and a common protocol [6]. Table 1 contains the FDA Reference Panel results of widely utilized lab-based and rapid NAATs.

The low LOD of the Accula RT-PCR test places it among the best-in-class of lab-based tests for SARS-CoV-2 RNA detection. Assays with higher LODs will miss more infected individuals. One study estimated that each 10-fold increase in LOD is expected to increase the false negative rate by 13%, missing an additional 1 in 8 infected persons [7]. Notably, the Accula SARS-CoV-2 Test from Thermo Fisher Scientific had the lowest measured LOD among all rapid NAATs and rivals the most sensitive lab-based tests.

The Accula test utilizes proprietary PCR technology that enables shortened cycling times without the need for costly thermal cycler hardware and optical detection systems used in lab-based RT-PCR. Testing is fully integrated on a single-use cassette and reusable dock, and results are provided in a lateral flow readout (similar to a home pregnancy test) in approximately 30 minutes. In field-based testing, the Accula test has also demonstrated sensitivity on par with standard lab-based RT-PCR.

### Rapid Ag tests

No FDA Reference Panel exists for Ag tests, so it is challenging to utilize the LOD data from package inserts to predict the clinical sensitivity of such tests, since a variety of reference materials and methodological approaches have been used by the manufacturers. However, reports of rapid Ag test performance among asymptomatic individuals in real-world settings are now available (Table 2). These studies [8-12] include school- and community-based screening of children and adults, as well as testing of close contacts of index cases, with prevalence (RT-PCR positivity rates) ranging from low (2%) to high (>15%). Sensitivity in the field (i.e., positive percent agreement (PPA) with lab-based RT-PCR results) ranged from ~35% to 63% across different rapid Ag tests and clinical contexts. In general, performance improved at higher prevalence and/or clinical risk (i.e., exposure to an infected individual). However, the ability to detect infections in asymptomatic individuals was suboptimal across all rapid Ag test studies. PPA of less than 80% between a rapid test and standard RT-PCR is considered poor performance by the FDA [13].

**Table 1. FDA Reference Panel results for widely utilized lab-based and rapid NAATs.**

	Limit of detection (NDU/mL*)	Lab-based test	Developer	Type of NAAT
Lab-based NAAT	600	Panther Fusion SARS-CoV-2 Assay	Hologic	RT-PCR
	1,800	cobas SARS-CoV-2 assay	Roche Molecular Systems	RT-PCR
	1,800	Quest SARS-CoV-2 rRT-PCR	Quest Diagnostics	RT-PCR
	2,700	Abbott RealTime SARS-CoV-2 assay	Abbott Molecular	RT-PCR
Rapid NAAT	475	Accula SARS-CoV-2 Test	Thermo Fisher Scientific	RT-PCR
	5,400	Xpress Xpert SARS-CoV-2 test	Cepheid	RT-PCR
	54,000	Visby Medical COVID-19	Visby Medical	RT-PCR
	60,000**	Cue COVID-19 test	Cue Health	RT-isothermal
	300,000**	ID NOW COVID-19 test	Abbott Diagnostics Scarborough	RT-isothermal

\* NDU: NAAT-detectable units.

\*\* Evaluated with dry swab protocol.

## Relationship between infectivity and test positivity

Proponents of rapid Ag tests suggest they may be at least as good as RT-PCR in the early phase of infection, when viral load and infectivity are highest. This argument is based on observations that positive Ag tests show high concordance with positive virus culture, while RT-PCR tests may continue to detect the presence of viral RNA after viable virus is no longer recovered in culture from patient specimens [14].

The implication is that samples that are positive by RT-PCR but negative by Ag test were likely sampled at the tail end of infections, with low viral loads unlikely to be infectious [15]. However, rapid Ag tests can give false negative results for samples with high viral loads as well.

In an example from the study of two community-based testing sites in Table 2, there were 79 instances where a sample was positive by lab-based RT-PCR and negative by the rapid Ag test. Fifty-one (51) of these rapid Ag false-negatives were available to be evaluated by virus culture. The majority of those samples were negative by virus culture, but six were positive.

Viral culture in artificial systems can have limitations [12], including “notoriously poor analytical sensitivity” [16]. The absence of culturable virus does not necessarily indicate the absence of transmissible virus, and the viral load below which transmissions no longer take place is yet unknown. Thus, the inability to detect culturable virus should not be interpreted to mean that a person is not infectious.

**Table 2. Performance of rapid Ag tests on asymptomatic individuals.**

Test (developer)	Setting	Sample size	Population	Prevalence (%)	Pretest probability*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Sofia (Quidel) [8]	Two university campuses	871	Asymptomatic	2	Low	41.2	98.4	33.3	98.8
BinaxNOW (Abbott) [9]	Two community-based testing sites	2,592	Asymptomatic	4.7	Low	35.8	99.8	91.7	96.9
BinaxNOW (Abbott) [10]	Community drive-through testing site	829 974	Asymptomatic/ pediatric Asymptomatic/ adult	12.9 5.9	Moderate to high	65.4 70.2	99 99.6	90.9 90.9	95.1 98.1
CareStart (Access Bio) [11]	Community drive-through testing site	221 1,036	Asymptomatic/ pediatric Asymptomatic/ adult	16.7 12.4	High	51.4 50	97.8 99.1	82.6 88.9	90.9 93.3
BD Veritor (BD) [12]	Close contacts of index cases	2,678	Pre-/ asymptomatic	8.7	Substantial	63.9	99.6	94.3	96.7

\* Pretest probability considers both the prevalence of the target infection in the community and the clinical context of the individual being tested. If the prevalence of infection in the community is high, and the person being tested is symptomatic, then the pretest probability is generally considered high. If the prevalence of infection in the community is low, and the person being tested is asymptomatic and has not had any known contact to a person with SARS-CoV-2, then the pretest probability is generally considered low. The generic grading here follows the thresholds proposed by the CDC [3]. PPA: positive percent agreement; NPA: negative percent agreement.

## **Guidance for asymptomatic screening**

United States (US) federal entities and professional organizations continue to review the evidence supporting rapid SARS-CoV-2 screening of asymptomatic individuals. Current guidelines carefully consider the advantages of rapid tests—quick turnaround time, lower costs, and resource needs—in the context of potential limitations of performance.

**Note:** Most EUA-authorized SARS-CoV-2 molecular diagnostic tests have been authorized for use in individuals suspected of COVID-19 by their health care providers. Individuals suspected of COVID-19 infection or exposure can be symptomatic, presymptomatic, or asymptomatic. Testing of any of these individuals is at the discretion of the health care provider ordering the test [17].

## **US Food and Drug Administration (FDA)**

The FDA recommends using a highly sensitive test for asymptomatic screening, especially if rapid turnaround times are possible. If highly sensitive tests are not feasible or if turnaround times are prolonged, the use of less sensitive point-of-care tests may be implemented, with consideration given to serial use to help mitigate performance deficits. When less sensitive tests are used, “negative” results should be considered “presumptive negative” [17].

## **US Centers for Disease Control and Prevention (CDC)**

The CDC views point-of-care serial screening as an important tool to identify asymptomatic cases when community risk or transmission levels are substantial or high [3]. In their guidance for rapid testing [18], screening tests are recommended on at least a weekly basis, given that the virus incubation period can be up to 14 days. If prevalence is high, more frequent screening might be needed. In most cases, negative Ag test results should be considered presumptive, meaning that they are preliminary results. For positive results from rapid Ag tests, especially with low pretest probability, confirmatory RT-PCR testing is recommended. Results from NAATs are considered the definitive result when there is a discrepancy between the Ag and NAAT tests.

## **Infectious Diseases Society of America (IDSA)**

According to a publication on strategies for SARS-CoV-2 testing [16], the IDSA recommends a testing regimen for asymptomatic individuals in settings of high prevalence with increased transmission risk and/or higher likelihood of severe disease. Examples include densely staffed workplaces, congregate settings, and cohorts with high rates of medical comorbidity, such as manufacturing and agricultural factories, inpatient psychiatric facilities, long-term acute care hospitals, and long-term care facilities. In these settings, the danger of missing a diagnosis includes the risk to the individual and the risk of missing a sudden local spike in infections at an early stage. When resources permit, such facilities should follow regional incidence numbers and initiate broad test-based screening when local prevalence reaches a predefined threshold (e.g., 1% test positivity). Screening would ideally occur at least twice weekly, with results available within 24 hours. The choice of tests depends on relative sensitivity, specificity, turnaround time, operational complexity, and cost. While NAATs have higher sensitivity, rapid Ag tests could be useful for frequent screening. If suspicion of SARS-CoV-2 infection is high, negative rapid Ag tests or isothermal NAATs should be confirmed with standard or rapid RT-PCR [4].

## **Conclusion**

Decentralized screening for SARS-CoV-2 infection is an essential tool in our recovery from the current crisis. Evidence to date strongly points to rapid RT-PCR tests as the optimal solution for asymptomatic screening, based on performance characteristics equivalent to gold-standard lab-based testing for SARS-CoV-2 and operational characteristics enabling rapid, decentralized deployment. Ag tests are less sensitive (more false negative results) compared to NAATs, especially among asymptomatic people. However, rapid isothermal or Ag tests may be useful when PCR tests are not available, taking into account performance limitations. Screening using less sensitive tests can be particularly helpful when testing is done serially and in areas with substantial or high levels of community transmission. Rapid RT-PCR such as the Accula SARS-CoV-2 Test is an ideal solution that addresses the requirements for timely results with high sensitivity and specificity.

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