Rapid diagnostic testing

Rapid detection of SARS-CoV-2: a comparison of test performance for rapid NAAT and antigen tests

Key messages

- Point-of-care testing for SARS-CoV-2 infection is an essential tool in our recovery from the SARS-CoV-2 pandemic.
- Rapid tests that detect viral antigens and RNA are available but must be implemented with consideration for potential limitations in performance.
- Rapid RT-PCR testing is an ideal solution that addresses requirements for timely results, and is often capable of sensitivity and specificity comparable to lab-based PCR.

Introduction

SARS-CoV-2 testing remains an essential tool as we seek to restore the health of communities globally. Lab-based nucleic acid amplification tests (NAATs) that use reverse-transcription polymerase chain reaction (RT-PCR) technology can be highly sensitive and specific for detecting SARS-CoV-2 RNA [1]. However, standard lab-based RT-PCR relies on specialized facilities 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 testing 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, and rapid tests including NAATs and antigen 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**—results in <1 hour
- **Cost benefits**—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 to dispel some common misconceptions regarding rapid tests—focusing on the science and regulatory guidance.

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 (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. Ag tests do not amplify their protein targets, so they are generally less sensitive than most NAATs (rapid or standard) [2]. While performance will vary from test to test, the generally lower sensitivity Ag tests has prompted the FDA to recommend repeat (or serial) testing for negative Ag test results to reduce the risk of false negative results [3].

NAATs

Tests within each category (rapid NAAT or rapid Ag) do not have equivalent performance and should be evaluated on an individual basis. Among rapid NAATs, there are several ways to amplify and detect the viral RNA. Rapid RT-PCR systems use temperature cycling to generate many copies from a single molecule. Isothermal amplification systems do not require the sophisticated thermal cycling involved in RT-PCR but are often less sensitive than rapid RT-PCR [4]. The FDA established a reference panel for SARS-CoV-2 NAATs, enabling direct comparison of limit of detection (LOD) across Emergency Use Authorization (EUA) tests, utilizing standardized material and a common protocol [5,6]. Table 1 shows the FDA reference panel results of widely utilized lab-based and rapid NAATs [5,6].

The LOD for rapid NAATs ranges from 475 to 300,000 NDU/mL. More specifically, the LOD for rapid RT-PCR tests is even lower, ranging from 475 to 54,000 NDU/mL. Assays with higher LODs will miss more infected individuals, thus exhibiting lower performance. There are rapid RT-PCR tests that have demonstrated sensitivity on par with standard lab-based RT-PCR tests [7-9].

Rapid Ag tests

Unlike for NAATs, the FDA does not have a reference panel for Ag tests. Since a variety of reference materials have been used by manufacturers to determine LOD, it is difficult to compare sensitivity based on reported LODs. However, reports of rapid Ag test performance compared to RT-PCR tests in real-world settings are now available. The studies, summarized in Table 2, include school- and community-based testing 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%). Across these studies the rapid Ag tests showed high specificity (~85–100% negative percent agreement with lab-based RT-PCR results). Sensitivity in the field (positive percent agreement (PPA) with lab-based RT-PCR results) ranged from ~35% to 86%, and positive predictive value (PPV) ranged from 33% to 100%, across different rapid Ag tests and clinical contexts [10-13]. In general, sensitivity improved at high pretest probability (i.e., higher prevalence and/or clinical risk exposure to an infected individual). However, the ability to detect infection in presymptomatic or asymptomatic individuals was suboptimal (<80%) across all rapid Ag test studies, as summarized in Table 2.

Several studies show differences in viral load dynamics in vaccinated vs. unvaccinated individuals, with fully vaccinated individuals showing delayed peak viral loads and accelerated viral clearance [14,15]. As most of the US has either infection- or vaccine-induced antibodies against SARS-CoV-2 [16], this will additionally lower the pretest probability across the population.

Table 1. FDA reference panel results for widely used lab-based and rapid NAATs [5,6].

	Limit of detection (NDU/mL)*	Molecular 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 RT-PCR test	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	Xpert™ Xpress SARS-CoV-2 test	Cepheid	RT-PCR
	54,000	Visby Medical™ COVID-19 test	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.

A recent study by Chu et al. [17] performed daily testing comparing rapid Ag and lab-based RT-PCR detection. Ag detection peaked at 3 days after illness onset for symptomatic cases and 3 days after the first positive RT-PCR test for asymptomatic cases, detecting 80% of symptomatic and 50% of asymptomatic cases, with consistently lower positivity rates for vaccinated than for unvaccinated cases. This study highlights the impacts of multiple pretest factors on SARS-CoV-2 detection and the importance of repeat testing for rapid Ag negatives.

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 [18].

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 [19]. 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, listed in Table 2, there were 79 instances where a sample was positive by a lab-based RT-PCR test and negative by a rapid Ag test. Of these rapid Ag false-negative samples, 51 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 [13], including "notoriously poor analytical sensitivity" [20]. The absence of culturable virus does not necessarily indicate the absence of transmissible virus, and the viral load below which transmission no longer takes 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 compared to RT-PCR tests.

Test (developer)	Setting	Sample size	Population	Prevalence (%)	Pretest probability*	Sensitivity (%)	Specificity (%)	PPV** (%)	NPV** (%)
Sofia™ test (Quidel) [10]	Two university campuses	871	Asymptomatic	2.0	Low	41.2	98.4	33.3	98.8
		227	Symptomatic	17.6	High	80.0	98.9	94.1	95.9
BinaxNOW™ test (Abbott) [11]	Two community-based testing sites	2,592	Asymptomatic	4.7	Low	35.8	99.8	91.7	96.9
		827	Symptomatic	21.3	High	64.2	100.0	100.0	91.2
CareStart™ test (Access Bio) [12]	Community drive-through testing site	221	Asymptomatic/ pediatric	16.7	High	51.4	97.8	82.6	90.9
		1,036	Asymptomatic/ adult	12.4	High	50.0	99.1	88.9	93.3
		27	Symptomatic for ≤7 days, pediatric	25.9	High	85.7	85.0	66.6	94.5
		169	Symptomatic for ≤7 days, adult	30.2	High	84.3	97.5	93.5	93.5
BD Veritor™ test (BD) [13]	Close contacts of index cases	2,678	Asymptomatic or presymptomatic	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 with 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].

** PPV: positive predictive value; NPV: negative predictive value.

Guidance for diagnostic testing

US federal entities and professional organizations continue to review evidence and update recommended testing algorithms. Current guidelines carefully consider the advantages of rapid tests—quick turnaround time, lower costs, and resource needs—in the context of potential limitations in performance.

Note: Most EUA-authorized SARS-CoV-2 molecular diagnostic tests have been authorized for use on individuals suspected of having COVID-19 by their health care providers. Testing of any of these individuals is at the discretion of the health care provider ordering the test [21].

US Food and Drug Administration (FDA)

The FDA regards antigen tests as less sensitive and therefore less likely than NAATs to pick up very early infections [3]. Consequently, the FDA recommends repeat testing on all negative Ag tests whether the individual is displaying symptoms or not. For persons experiencing symptoms or testing due to known exposure, NAAT testing is recommended following a negative Ag test [21]. If NAAT testing is not available, serial testing should be performed within 1–2 days for persons with COVID-19 symptoms; persons with known exposure should test 3 times with 48 hours between tests [3]. Positive Ag test results do not need to be confirmed.

US Centers for Disease Control and Prevention (CDC)

The CDC recommends testing for people with symptoms or who have had close contact with someone with COVID-19. In addition, the CDC views point-of-care serial screening as critical to slowing the spread of SARS-CoV-2 when community levels are medium to high, particularly in workplaces, schools, and other community settings (e.g., nursing homes, shelters, and correctional facilities) [1]. Negative Ag tests in persons with signs or symptoms of COVID-19 should be confirmed by an NAAT, a more sensitive test. For positive results from rapid Ag tests, especially with low pretest probability, confirmatory RT-PCR testing is recommended. Results from NAATs are generally considered definitive when there is a discrepancy between the Ag and NAAT tests [22].

Infectious Diseases Society of America (IDSA)

The IDSA recommends either rapid RT-PCR or standard lab-based NAATs over isothermal NAATs for symptomatic individuals [4]. Patients with high clinical suspicion of COVID-19 who have a negative NAAT result should be retested within 24–48 hours, and clinicians should review symptoms to confirm appropriate specimen type. The IDSA recognizes the higher sensitivity of molecular diagnostics over Ag testing. When NAAT testing is not feasible, Ag testing can be used without confirming positive results. However, negative results should be confirmed by standard NAAT when clinical suspicion or community impact is high [4].

Conclusions

Point-of-care testing for SARS-CoV-2 infection is an essential tool in our continued recovery from the SARS-CoV-2 crisis. Evidence to date strongly points to rapid RT-PCR tests as the optimal solution for testing, with many showing performance characteristics comparable to lab-based RT-PCR testing for SARS-CoV-2 and operational characteristics enabling rapid, decentralized deployment. Ag tests are less sensitive (increased risk of false negative results) than NAATs. However, Ag tests may be useful when rapid NAAT tests are not available, but performance limitations must be taken into account. Testing 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 is an ideal solution that addresses the requirements for timely results with sensitivity and specificity comparable to lab-based PCR.

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The Accula SARS-CoV-2 test has not been FDA cleared or approved but has been authorized for emergency use by the FDA for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high, moderate, or waived complexity tests. The test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.