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Rapid Antigen Assays for SARS-CoV-2: Promise and Peril



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KEYWORDS

• SARS-CoV-2 • COVID-19 • Rapid antigen testing

KEY POINTS

- Rapid antigen detection tests (RADTs) for SARS-CoV-2 are most likely to be positive when viral loads are higher.
- The diagnostic performance characteristics of RADTs vary widely among different test brands, patient populations, and symptomatic status.
- RADTs enable more widescale scaling-up of testing globally. However, they are not appropriate as a test to determine infectiousness.
- In practice, the use of antigen testing to screen asymptomatic persons appears to work best when combined with other public health measures and not as a sole intervention method.

INTRODUCTION

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) initially caught many countries on the backfoot. The trials and tribulations of the initial roll-out of coronavirus disease 2019 (COVID-19) testing in the United States are well documented.¹ Although the specifics are beyond the scope of this article, they were numerous and well-publicized: from unavailability of swabs, reagents, and pipette tips to regulatory hurdles, initial testing stumbled in the United States in the face of a global pandemic caused by a novel virus.

Although reverse transcription–polymerase chain reaction (RT-PCR) and transcription-mediated amplification (TMA, hereafter PCR) remain the gold standard for the detection of SARS-CoV-2, at the time of writing, such testing still remains predominantly confined to diagnostic laboratories.² In contrast, rapid antigen detection tests (RADTs) lend themselves to widespread deployment because of their relatively

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low cost, and in many cases, the ability to be run without the use of specialized equipment.³ This makes testing possible for clinics, geographic areas without proximal access to laboratories, and even in one's own home. RADTs have the potential to relieve pressure on diagnostic laboratories, who even well into 2022, continue to face labor and supply chain constraints. They represent a chance at test scalability beyond what can reasonably be achieved by diagnostic laboratory-based testing models.

SARS-CoV-2 viral loads are similar between asymptomatic and symptomatic cases and it was recognized relatively early in the pandemic that asymptomatic individuals are an important epidemiologic driver of spread.⁴ Infrequent recovery of SARS-CoV-2 in culture after the first week of symptoms in mild-to-moderate cases, as well as the relatively high correlation between positive antigen test results and viral culture, led to the perception that RADTs could serve a role in assessing the presence of infectious virus.^{5,6} Antigen testing has been touted as key in "returning to normal," with the concept of knowing ones "infectiousness status" having been widely popularized. Nevertheless, this concept is not without controversy or scientific counter-argument. Here, we discuss the utility of SARS-CoV-2 antigen testing, the unique opportunities it presents, and important considerations surrounding its use.

BACKGROUND

Before the COVID-19 pandemic, RADTs for respiratory viruses were historically associated with suboptimal test performance. This was dramatically illustrated during the emergence of novel H1N1 influenza A, where sensitivities of RADTs were found to be as low as 17.8%.⁷ Meta-analyses evaluating the performance of influenza RADTs showed pooled sensitivities of 64.6% (95% confidence interval [CI], 59.0%–70.1%) and 52.2% (95% CI, 45.0%–59.3%) for influenza A and B, respectively, with pooled specificities of 98.2% (95% CI, 97.5%–98.7%).⁸ Subsequent refinements such as the use of digital readers led to tests with improved sensitivities of up to 80%,⁹ nevertheless, RADTs for influenza are less sensitive than molecular methods, with the Centers for Disease Control and Prevention (CDC) recommending confirmatory testing of negative specimens when influenza infection is suspected.^{10,11}

Important differences between SARS-CoV-2 and other respiratory viruses inform how we might think about the potential utility of antigen testing for COVID-19. Although asymptomatic transmission was initially thought to be responsible for more than half of all COVID-19 cases, more recent estimates range between 17% and 30%.¹² In contrast, rates of asymptomatic influenza transmission are much lower.¹³ Interestingly, influenza viral loads are 1 to 2 log₁₀ copies lower among asymptomatic individuals, which may account in part for these lower transmission rates, though other studies refute this.^{14,15} To prevent transmission of SARS-CoV-2 in hospital settings, many facilities in the United States perform PCR before surgery or other procedures (eg, colonoscopy), and even upon hospital admission.^{16,17} Such testing has thus further compounded test demand for SARS-CoV-2 in traditional diagnostic laboratory settings. Arguably never before in the history of laboratory medicine has there been such high diagnostic testing demand for one agent of infection. It is into this landscape that massive interest in antigen testing for COVID-19 has emerged.

"Can I HAVE 'What IS THE REFERENCE METHOD? FOR \$800"?

PCR is widely recognized as the most sensitive method to diagnose respiratory viral infections, having almost entirely replaced other previously commonly used test methods (ie, viral culture, direct fluorescence, etc). Nevertheless, an unfortunate and erroneous perception has emerged among the general public that PCR lacks

specificity for SARS-CoV-2 detection. This stems from observations of SARS-CoV-2 RNA detectability for extended periods, long after resolution of infection.⁴ Cycle threshold (Ct) values are typically high (>35) in such cases (ie, generally above the assay's limit of detection [LoD]) and repeat testing in these cases, therefore, oscillates between detection and nondetection.¹⁸ High Ct values themselves do not necessarily indicate resolved infection, as such values can also be observed early in the infection course (before an exponential increase in viral loads) as well as among patients admitted to hospital with symptomatic disease.¹⁹ Importantly, analytical false-positive SARS-CoV-2 PCR results have been shown to occur extremely rarely, though issues with specific assays have been observed.^{20–22}

RADTs detect viral protein, usually viral nucleocapsid in the case of SARS-CoV-2. Nasal and nasopharyngeal specimens are predominantly used, with throat and saliva performing poorly for several RADT brands.^{23–25} Chemiluminescent antigen tests may be compatible across all sample types.²⁶ Although commonly referred to in the popular press as if they are monolithic, there are countless different antigen test brands available worldwide, with 45 having received emergency use authorization (EUA) by the US Food and Drug Administration (FDA) at the time of writing.²⁷ Critically, not all brands perform equivalently: of 64 different antigen tests directly compared in one seminal study, only 29% met minimum performance standards defined as $\geq 97\%$ sensitivity and an LoD of 60% at 100 plaque-forming units per milliliter.^{28,29}

Regardless of the brand tested, some common principles have emerged: (1) SARS-CoV-2 RADTs are more likely to be positive when viral loads are higher, and (2) they are less likely to be positive after the first week of symptoms. High rates of antigen positivity are generally observed when Ct values are lower (ie, higher quantities of viral RNA detected), and when it is still possible to isolate infectious (ie, replication-competent) viruses *in vitro*.³⁰ For example, although sensitivity of the Innova assay was 78.2% overall in one study, sensitivity was 97% for specimens with Ct values < 25, and 95.5% for Ct values less than 28.³¹ Similar findings have been observed for many assays across a multitude of studies.^{28,32} It is important to recognize that Ct values vary widely and differ enormously between assays and laboratories.³³ Furthermore, Ct values are dramatically impacted by specimen quality and can be artificially high for poor-quality specimens, with poor specimen collection contributing to falsely negative results.³⁴

Detection of culturable virus peaks near the time of symptom onset, with the probability of isolating infectious virus reduced to 6% after 10 days of symptoms among patients with mild-moderate infection.³⁵ Low rates of viral culture positivity have generally been observed for upper respiratory tract specimens with high Ct values,³⁵ but isolation of infectious virus has been observed for extended periods among patients with severe-to-critical illness³⁶ and among adults and children with prolonged severe immune compromise.^{37,38} Although viral culturability is related to Ct value, cytopathic effects were observed among 43% of patients with severe infection and Ct values ≥ 26 , compared with 22% for mild-to-moderate cases and even from specimens with Ct values ≥ 35 (5% in mild cases, 15% in severe cases).³⁹ Consequently, the relationship between high Ct values and the absence of culturability is not absolute; viral culturability can and does occur among specimens with high Ct values, albeit less frequently.

The high correlation between culture and antigen positivity has led to the concept of RADTs as an “infectiousness test.” It has been proposed that viral culture may therefore be a more appropriate reference standard than PCR for asymptomatic testing. Nevertheless, this assumption is problematic as viral culture studies for SARS-CoV-

2 published to date lack standardization.⁴⁰ Preculture specimen handling (eg, storage at 4°C), as well as the cell line and culture protocol used, greatly impact the performance of culture.⁴¹ Thus, although culture positivity indicates the presence of infectious virus, the absence of culture positivity itself does not definitively rule out the presence of infectious virus.

DIAGNOSTIC PERFORMANCE IN SYMPTOMATIC POPULATIONS

Performance characteristics of RADTs compared with PCR vary in published studies, likely due to differences in study design, PCR assay used, participant recruitment and demographics, and symptom duration. Although some RADT brands show solid performance characteristics, meta-analyses show a wide range of sensitivities among symptomatic patients (68.9% average sensitivity; 95% CI, 61.8%–75.1%).⁴² Antigen testing specificity is generally high (99.6%; 95% CI, 99.0%–99.8%), though clusters of false-positive results have been described.⁴³ A summary of test performance among common brands is shown in **Table 1**.

Interpretation of studies regarding the performance of antigen testing among symptomatic patients can be challenging, even for assays considered to be among the most sensitive on the market. For example, among symptomatic participants at a community testing site in Wisconsin, the sensitivity of the Abbott BinaxNOW Ag Card (BinaxNOW) lateral flow immunoassay was 78.6% (95% CI, 73.4%–83.3%) compared with RT-PCR,⁴⁴ but was 95.4% (95% CI, 90.2%–98.3%) among symptomatic participants at a community testing site based in San Francisco⁴⁵ and 94.1% (95% CI, 71.1%–100%) at a similar site in the Netherlands.⁴⁶ Although some studies show high sensitivity for antigen testing in hospital settings, other studies have reported sensitivities as low as 66.4% (95% CI, 57.0%–74.9%).⁴⁷

For assays that use digital readers, the Quidel SOFIA SARS Antigen Fluorescent Immunoassay (FIA) had 80.0% sensitivity (95% CI, 64.4%–90.9%) and 98.9% specificity (95% CI, 96.2%–99.9%) among symptomatic participants at 2 university campuses in Wisconsin, lower than what was reported in the FDA EUA.⁴⁸ A different study observed an 82% positive agreement with RT-PCR among symptomatic patients at an urgent care center.²⁸ The FIA also had comparable performance to the BD Veritor (Veritor) chromatographic digital immunoassay antigen test in specimens collected from drive-through and outpatient sites, despite apparent differences in sensitivity according to their respective EUA submission data.⁴⁹

It is generally accepted that RADTs are unlikely to be positive beyond 7 days of symptoms, though this can differ somewhat by RADT brand (ie, 5 days vs 7 days). For example, the Innova assay remained positive for several days longer than the SureScreen F assay in one study.³¹ Test performance may also be influenced by the number of symptoms present. For instance, the Veritor assay had 66.7% (95% CI, 30.0%–90.3%) positive agreement with PCR for patients with one symptom within 5 days of onset, compared with 88.0% (95% CI, 70.0%–95.8%) agreement among patients with at least 2 symptoms.⁴⁹

RADT performance among pediatric patients has been variable between studies. Slightly lower concordance with RT-PCR was observed among children compared with adults (82% vs 85%, respectively).⁵⁰ Among symptomatic hospitalized kids, one study reported greater than 95% sensitivity for the PanBio assay,⁵¹ but a much lower sensitivity of 45.4% (95% CI, 34.1%–57.2%) was observed in a different large multihospital study.⁵² These divergent results may reflect differences in performance in adults versus children, regional differences in patient population, or differences in PCR methodology.

Table 1
Currently available FDA-cleared SARS-CoV-2 antigen tests²⁷

Manufacturer	Test Name	Technology Used	Home-Use Option ^a	Indicated for Asymptomatic Testing	Separate Instrument Required	Performance Data for Detection of SARS-CoV-2
Abbott Diagnostics Scarborough, Inc	BinaxNOW COVID-19 Ag Card	Lateral flow immunoassay	No	No	No	PPA: 84.6% (76.8%–90.6%) NPA: 98.5% (96.6%–99.5%)
Abbott Diagnostics Scarborough, Inc	BinaxNOW COVID-19 Ag Card Home Test	Lateral flow immunoassay	Yes	No	Smartphone	PPA: 91.7% (73.0%–98.9%) NPA: 100.0% (87.7%–100.0%)
Abbott Diagnostics Scarborough, Inc	BinaxNOW COVID-19 Antigen Self Test	Lateral flow immunoassay	Yes	Yes	No	PPA: 91.7% (73.0%–98.9%) NPA: 100.0% (87.7%–100.0%)
Abbott Diagnostics Scarborough, Inc	BinaxNOW COVID-19 Ag Card 2 Home Test	Lateral flow immunoassay	Yes	Yes	Smartphone	PPA: 91.7% (73.0%–98.9%) NPA: 100.0% (87.7%–100.0%)
Abbott Diagnostics Scarborough, Inc	BinaxNOW COVID-19 Ag 2 Card	Lateral flow immunoassay	No	Yes	No	PPA: 84.6% (76.8%–90.6%) NPA: 98.5% (96.6%–99.5%)
Access Bio, Inc.	CareStart COVID-19 Antigen test	Lateral flow immunoassay	No	Yes	No	PPA: 93.75% (79.85%–98.27%) NPA: 99.32% (96.27%–99.88%)
ACON Laboratories, Inc.	Flowflex COVID-19 Antigen Home Test	Lateral flow immunoassay	Yes	Yes	No	PPA: 93% (81%–99%) NPA: 100% (97%–100%)
Becton, Dickinson and Company (BD)	BD Veritor System for Rapid Detection of SARS-CoV-2	Lateral flow immunoassay	No	Yes	No	PPA: 84% (67%–93%) NPA: 100% (98%–100%)
Becton, Dickinson and Company (BD)	BD Veritor System for Rapid Detection of SARS-CoV-2 & Flu A + B	Lateral flow immunoassay	No	No	No	PPA: 86.7% (75.8%–93.1%) NPA: 99.5% (97.4%–99.9%)

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Table 1
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Manufacturer	Test Name	Technology Used	Home-Use Option^a	Indicated for Asymptomatic Testing	Separate Instrument Required	Performance Data for Detection of SARS-CoV-2
Celltrion USA, Inc.	Celltrion DiaTrust COVID-19 Ag Rapid Test	Lateral flow immunoassay	No	Yes	No	PPA: 93.3% (78.7%–98.2%) NPA: 99.0% (94.7%–99.8%)
Celltrion USA, Inc.	Sampinute COVID-19 Antigen MIA	Magnetic force-assisted electrochemical sandwich immunoassay	No	No	Yes	PPA: 94.4% (80.0%–99.0%) NPA: 100.0% (88.0%–100.0%)
DiaSorin, Inc.	LIAISON SARS-CoV-2 Ag	Chemiluminescent immunoassay	No	No	No	PPA: 97.0% (84.7%–99.5%) NPA: 100% (96.6%–100%)
Ellume Limited	Ellume COVID-19 Home Test	Lateral flow immunoassay	Yes	Yes	No	PPA: 95% (82%–99%) NPA: 97% (93%–99%)
iHealth Labs, Inc.	iHealth COVID-19 Antigen Rapid Test Pro	Lateral flow immunoassay	No	No	No	PPA: 88.2% (73.4%–95.3%) NPA: 100% (88.6%–100%)
iHealth Labs, Inc.	iHealth COVID-19 Antigen Rapid Test	Lateral flow immunoassay	Yes	Yes	No	PPA: 94.3% (81.4%–98.4%) NPA: 98.1% (93.3%–99.5%)
InBios International, Inc.	SCoV-2 Ag Detect Rapid Test	Lateral flow immunoassay	No	Yes	No	PPA: 86.67% (73.82%–93.74%) NPA: 100% (98.53%–100.00%)
Luminostics, Inc.	Clip COVID Rapid Antigen Test	Lateral flow immunoluminescent assay	No	No	Yes	PPA: 96.96% (83.8%–99.9%) NPA: 100% (97.3%–100%)
LumiraDx UK Ltd.	LumiraDx SARS-CoV-2 Ag Test	Microfluidic immunofluorescence assay	No	No	Yes	PPA: 97.6% (91.6%–99.3%) NPA: 96.6% (92.7%–98.4%)
OraSure Technologies, Inc.	IntelliSwab COVID-19 Rapid Test Rx	Lateral flow immunoassay	Yes	No	No	PPA: 84% (71%–92%) NPA: 98% (93%–99%)

OraSure Technologies, Inc.	IntelliSwab COVID-19 Rapid Test	Lateral flow immunoassay	Yes	Yes	No	PPA: 84% (71%–92%) NPA: 98% (93%–99%)
OraSure Technologies, Inc.	IntelliSwab COVID-19 Rapid Test Pro	Lateral flow immunoassay	No	Yes	No	PPA: 84% (71%–92%) NPA: 98% (93%–99%)
Ortho Clinical Diagnostics, Inc.	VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack	Chemiluminescent immunoassay	No	No	Yes	PPA: 80.0% (56.6%–88.5%) NPA: 100.0% (95.2%–100.0%)
Princeton BioMeditech Corp	Status COVID-19/Flu	Lateral flow immunoassay	No	No	No	PPA: 93.9% (83.5%–97.9%) NPA: 100% (95.2%–100%)
Qorvo Biotechnologies, LLC	Omnia SARS-CoV-2 Antigen Test	Bulk acoustic wave biosensor	No	No	Yes	PPA: 89.47% (78.88%–95.09%) NPA: 100% (89.28%–100%)
Quanterix Corporation	Simoa SARS-CoV-2 N Protein Antigen Test	Paramagnetic microbead-based immunoassay	No	No	Yes	PPA: 97.7% (92.03%–99.72%) NPA: 100% (90.75%–100.0%)
Quidel Corporation	Sofia SARS Antigen FIA	Lateral flow immunofluorescence assay	No	Yes	Yes	PPA: 96.7% (83.3%–99.4%) NPA: 100% (97.9%–100.0%)
Quidel Corporation	Sofia 2 Flu + SARS Antigen FIA	Lateral flow immunofluorescence assay	No	No	Yes	PPA: 95.2% (84.2%–98.7%) NPA: 100.0% (96.9%–100.0%)
Quidel Corporation	QuickVue SARS Antigen Test	Lateral flow immunoassay	No	No	No	PPA: 96.6% (88.3%–99.0%) NPA: 99.3 (96.0%–99.9%)
Quidel Corporation	QuickVue At-Home COVID-19 Test	Lateral flow immunoassay	Yes	No	No	PPA: 84.8% (71.8%–92.4%) NPA: 99.1% (95.2%–99.8%)
Quidel Corporation	QuickVue At-Home OTC COVID-19 Test	Lateral flow immunoassay	Yes	Yes	No	PPA: 83.5% (74.9%–89.6%) NPA: 99.2% (97.2%–99.8%)
Salofa Oy	Sienna-Clarity COVID-19 Antigen Rapid Test Cassette	Lateral flow immunoassay	Yes	No	No	PPA: 87.5% (68.6%–93.0%) NPA: 98.9% (94.2%–99.9%)

^a Over-the-counter or prescription required.

Symptomatic testing is arguably the most high-stakes application for RADTs. Antigen testing has limited utility in the diagnosis of symptomatic COVID-19 in hospital settings because patients who require hospitalization can present for evaluation after more than 1 week of symptoms. For this reason, they have been deemed to be unsuitable for rapid “rule-out” in an emergency department setting.^{32,53} In nonhospital settings, however, the results of laboratory-based testing can take several days to return when demand is high, which makes it impractical for symptomatic people to isolate pending their test results. Thus, the primary benefit of a positive RADT result in symptomatic individuals in the community is the ability to facilitate immediate self-isolation when positive. However, low viral loads (and thus RADT-negativity) can occur during infection due to the natural course of illness, as well as due to suboptimal sample collection. Care must be taken to interpret tests in the context of symptoms and exposures, which both influence pretest probabilities. Finally, with the increasing prevalence of disease, it is important to remember that the negative predictive value of RADTs decreases.³ Thus, the CDC currently recommends negative antigen results in the presence of symptoms should be confirmed with a molecular-based test.⁵⁴

PERFORMANCE AS A TEST OF INFECTIOUSNESS IN ASYMPTOMATIC POPULATIONS

The ability of antigen testing to identify infectious asymptomatic or presymptomatic individuals, facilitating prompt self-isolation and breakage of transmission chains is an important application of this technology. RADT positivity drops steeply after infectious virus is no longer detected by cell culture, with a sensitivity of 23.8% at the end of 1 week of initial culture positivity compared with 85.7% for PCR in one study.³⁶ Antigen testing has therefore been suggested as a proxy for infectiousness; however, there is currently no infectiousness test for COVID-19 and the infectious dose of SARS-CoV-2 remains unknown. Detection of subgenomic (sg) RNA or minus-strand RNA indicates the presence of active viral replication and is generally interpreted as signifying that infectious virus may be present.⁵⁵ However, the relationship between sgRNA and culture positivity itself is somewhat controversial.⁵⁶ For better or worse, viral culture has become the *de facto* method for assessing infectiousness for COVID-19, with the limitations of viral culture having been discussed earlier.⁵⁷

A major assumption has been that false-negative RADT results represent previously infected individuals with prolonged low-level detection by PCR. However, positivity by PCR generally can also precede RADT positivity by approximately 1 to 2 days,³¹ and 75% of false-negative RADT results in one community study occurred among individuals who were presymptomatic.⁵⁰ In the same study, 11.8% of false-negative RADT specimens were culture positive with 53% being sgRNA positive. Thus, the proportion of false-negative RADT results that represent previous infection, rather presymptomatic infection (and thus potentially infectiousness), likely differs, depending on where in the epidemic curve a particular community is at that time because a greater proportion of people are early in the infection when cases are on the rise.⁵⁸

For antigen testing to have utility as an “infectiousness test,” one assumes that transmission is not generally readily observed among individuals with viral loads lower than what are reliably detected by RADTs. However, this does not necessarily appear to be the case. Firstly, significant overlap in Ct values occurs between spreaders and nonspreaders alike, including documented transmission by individuals with high Ct values.⁵⁹ Secondly, individuals with low viral loads, defined in one study as 1×10^6 copies/mL, had a secondary attack rate that was half that of individuals with high viral loads, but was still significant at 12%.⁶⁰ When the rate of local spread is high, the

detection rate of RADTs for specimens with lower viral loads could potentially miss a sizable portion of presymptomatic and asymptomatic cases.⁶¹

Importantly, although the LoD of the BinaxNOW COVID-19 Antigen Card (Abbott, Scarborough, Maine) was shown to be approximately 4.0×10^4 to 8.1×10^4 copies per swab,³⁰ the LoD for several other RADT brands were shown to be much higher, at 2.1×10^6 to 2.9×10^7 per swab.⁶² As such, transmission can and does occur among individuals with viral loads that could reasonably be expected to test negative by RADTs. Critically, transmission dynamics are influenced by several factors beyond solely viral load; these include the contact pattern (eg, duration of exposure, activity), host factors (eg, age), and the environment (eg, ventilation).⁶³ Thus, a negative RADT result in an asymptomatic individual should not be thought of as a definitive statement on noninfectiousness, but rather, indicating likely noninfectiousness at the time of sampling.

Initial modeling data suggested that more frequent serial testing can mitigate concerns over RADT sensitivity^{64,65} because the sensitivity of antigen testing before the first day of detectable shedding of infectious virus was 37.5% compared with 65% for RT-PCR. For this reason, FDA EUAs for asymptomatic testing specify that serial testing should be performed. Recently, 2 studies using real-world data have confirmed the results of initial modeling studies. In a large study among college students, weekly nasal PCR testing had a sensitivity of 98.7% for COVID-19 screening detection, with comparable sensitivities for RADTs (>98%) performed every third day, dropping to 79.7% when performed only weekly.⁶⁶ During an intercollegiate program of daily RADT and weekly paired PCR/antigen testing (81,175 and 23,462 tests, respectively), daily antigen testing had similar sensitivities to twice-to-thrice weekly PCR testing.⁶⁷

At face value, it could be argued asymptomatic testing has few downsides because any case detected is one that would otherwise have not been detected. From this perspective, the tests are viewed as a public health intervention rather than as a diagnostic, which was their intended design.^{68,69} However, false-negative test results have clinical consequences for the individual and can also influence the behavior of the test recipient in a fashion that promotes spread of the virus to others. A negative test result (whether PCR or antigen) is not a replacement for other public health measures and the same considerations that influence test performance in symptomatic individuals (eg, specimen quality, timing of testing) are similarly a consideration in this setting.

An additional concern is that even a test with excellent specificity will have a poor positive predictive value (PPV) during periods of low infection prevalence.^{70,71} For example, PPV is predicted to be only 28.8% for a test with 98% specificity when disease prevalence is 1%.⁷² This was also shown in a real-world study where a 66.7% false-positivity rate was noted for RADTs.⁴⁸ A different study performed in August 2020 reported that 23 of 39 (60%) of samples positive by RADT were negative by RT-PCR across several skilled nursing facilities.⁷³ The reasons for this cluster of false positives were never fully determined but could represent issues with poor quality test manufacturing. Concerns over test specificity can be mitigated by requiring positive results in asymptomatic people to be confirmed by secondary testing, which is recommended by the current CDC interim guidelines.⁵⁴ However, such confirmation may not necessarily be required when community infection rates are high. It is therefore important to continually reevaluate the prevalence of infection in a region where antigen testing will be implemented. Unfortunately, as in-home RADT becomes more available in the United States, a system for linkage to confirmatory PCR testing and to public health surveillance systems continues to be lacking, which leads to underestimates of disease prevalence.

ASYMPTOMATIC ANTIGEN TESTING IN PRACTICE

Several studies modeled the potential impact of widespread antigen testing to screen asymptomatic populations, with some even factoring in behaviors such as unwillingness to test or isolate.^{64,65,74} One study predicted that with a test sensitivity of 80%, weekly testing would prevent almost 3 million cases and over 16,000 deaths in the United States.⁷⁴ There are 5 main areas where asymptomatic antigen testing has been applied in practice and we will review each of these applications here.

Antigen Testing as a Circuit Breaker

Mass population testing has been implemented by some governments in an attempt to halt exponential increases in case numbers. RADT testing with swabs collected under supervision from military personnel was used in a large-scale community testing initiative over 6 months in the city of Liverpool. This intervention led to an estimated 18% increase (95% CI, 7%–29%) in case detection, a 21% reduction (95% CI, 12%–27%) in cases during the first 6 weeks compared with control areas, but had no significant impact on the number of hospital admissions.⁷⁵ Notably, in interviews with participants who chose not to test, the biggest barriers to participation were fear of income loss, skepticism over the need to test, as well as crowded and inconvenient waiting lines.⁷⁵

Arguably, it is Slovakia's experience that has driven the narrative about the power of mass antigen testing to halt case surges. The results from Slovakia are indeed striking: after one round of mass antigen testing, the prevalence of SARS-CoV-2 decreased by 58% (95 CI, 57%–58%). In districts that had a positivity rate $\geq 0.7\%$, a second round of testing was performed, after which a 0.3 decrease in the reproductive number (ie, R_0) occurred 2 weeks later.⁷⁶ Mass antigen testing revealed 5594 cases compared with only 782 cases that would have otherwise been identified by routine clinical testing. Disease prevalence decreased from 3.97% to 1% 1 week later with modeling data suggesting that 46,137 infections were prevented as a result of the program.⁷⁷

Importantly, mass antigen testing was not the sole public health intervention used by the Slovakian government; noncompliance in the testing program required a 10-day home quarantine, with random police inspections and a fine equivalent to 1.5 \times the national monthly wage issued for those caught breaking quarantine. Other measures implemented included limiting gatherings of more than 50 people and closure of restaurants and schools.⁷⁸ Though it was concluded that frequent mass testing would be necessary to sustain the decrease in cases, nevertheless, the Slovakian experience shows the potential of mass testing when combined with other measures. In practice, such measures would likely be considered unpalatable to a significant proportion of the US populace.

Daily Testing to Enable Close Contact

Daily antigen testing with the SOFIA assay (Quidel, San Diego, California), in addition to weekly PCR testing, was implemented by universities in the United States to facilitate the continuation of intercollegiate sports programs.⁶⁷ In all, 172 athletes had SARS-CoV-2 detected, with 52% identified as positive on days between weekly PCR tests. In all, 234 days of potential infectiousness were avoided as a result of the daily antigen testing program. There were also 98 false-positive RADT results, but negative impact was minimized by ready access to PCR results with rapid turnaround time.

The authors observed that antigen positivity lagged about 1 day behind PCR detection. Even with nurse-supervised specimen collection, daily antigen testing failed to prevent outbreaks on 2 occasions involving a total of 32 individuals.⁷⁹ PCR results

were available for the initial false-negative RADT result in 1 of the 2 index cases: despite a low Ct value of 15.9, the patient tested negative by RADT but subsequently developed symptoms later the same day. Mass PCR testing was performed 7 times during these outbreaks, leading to the identification of 21 new cases, 86% of whom were antigen negative. The most likely reason for the false-negative antigen test results in contact tracing networks is due to individuals being too early in their infection curve to be detectable by RADTs. In support of this, transmission was only interrupted when serial PCR was performed during the outbreak to identify additional cases. The study's authors concluded that "*serial antigen testing may have limited sensitivity for detecting early asymptomatic infections,*"⁷⁹ suggesting a two-tier strategy of antigen tests backed by intensive PCR sampling as the optimal approach for surveillance and containment.

To Attend Events or to Travel

The utility of day-of-testing to attend live, indoor concerts was investigated in 2 studies conducted in Catalonia, Spain. Attendees were tested using the Panbio assay (Abbott, Scarborough, Maine) before entry at a live music concert. Follow-up studies 8 days later showed no difference in COVID-19 incidence between the control and intervention groups.⁸⁰ Importantly, concertgoers were required to wear N95 face masks, the venue was well-ventilated, and there were restrictions on direction of movement and crowding in smaller spaces. In a follow-up study by the same group looking at a 5000-person event using the same combination of additional public health measures, 6 RADT positive and likely infectious individuals, along with 2 of their close contacts, were prevented from attending the concert.⁸¹ Only 6 cases were diagnosed within 2 weeks of the event, 3 of whom were identified as being close contacts of COVID-19 case who did not attend the concert. Although it is not possible to discern the specific impact of antigen testing per se, both studies showed that mass day-of-event RADT testing, when combined with masking and adequate ventilation, facilitates normalization of activity and mass gatherings.

Day-of-testing was also used to screen passengers at 2 major US airports before and immediately after their flights.⁸² Antigen testing detected 4 individuals (0.04%), who tested positive by both antigen testing and confirmatory RT-PCR.⁸² Concerningly, as infection prevalence was estimated to be low at 1.1% at the time, there were 12 false-positive rapid antigen tests results out of 9849 total tested that did not confirm by RT-PCR.⁸² This testing occurred in addition to other interventions already in place, for example, masking, vaccination, and distancing.

Postexposure Testing in Schools

RADTs have been tool to limit SARS-CoV-2 transmission in schools. In a randomized study involving secondary schools and colleges, students and staff with known exposure to an infected contact were either required to self-isolate for 10 days (control group) or given the option to continue attending school provided with daily RADT testing (intervention group).⁸³ Overall positivity postexposure was low at ~2% in both groups, and the impact of RADT alone is difficult to assess as other safety precautions were in place, for example, masking. School absences were also not significantly reduced in the intervention group.⁸³

Performance of At-Home Testing

Though some governments have provided at-home testing to their residents free-of-charge (eg, the United Kingdom and the United States), the overwhelming majority of studies to date on antigen testing involve performance by trained staff.^{45,84} The

performance of at-home RADTs is relatively understudied by comparison. Though studies show only minor differences in performance between health care worker (HCW)-collected and HCW-supervised specimen collection,⁸⁵ the performance of at-home, unsupervised specimen collection may differ. In a study looking at the performance of SARS-CoV-2 RT-PCR, home-collected specimens had a sensitivity of 80% compared with clinician-collected swabs⁸⁶; such differences would also be expected to apply to RADTs. Similarly, testing performed by laboratory scientists was 78.8% sensitive compared with fully trained research HCWs (70%), with both groups exceeding the sensitivity of self-trained members of the public (57.5%).²⁸ Rates of positivity were similar between the different collectors for specimens with low Ct values, but differences became more pronounced for specimens with Ct values in the 25–28 range.²⁸ Finally, one infrequently discussed element with regards to at-home testing is the potential for test transportation/shipping conditions to deleteriously impact test performance. False-negative results were noted when test kits stored at high temperatures, with false-positive results observed with lower temperatures.⁸⁷

CONCLUSIONS AND FUTURE CONSIDERATIONS

Variants

Antigen testing appears to thus far be unaffected by the emergence of SARS-CoV-2 variants at least with respect to the performance of the BinaxNOW (Abbott) assay, showing equivalent performance for major variants including the Delta variant (B.1.617)⁸⁸ and Omicron variant (B.1.429).^{89,90} The performance of other RADT brands is unknown as of the time of writing. Importantly, Bourassa and colleagues observed that SARS-CoV-2 specimens with a D399N nucleocapsid mutation were not detected by the SOFIA assay but were detected by the BinaxNOW test.⁹¹ Thus, continued investigation on the part of the RADT manufacturers and independent researchers will be necessary to ensure that a given RADT brand continues to perform equivalently as new variants continue to emerge.

Impact of Variants on Kinetics of Antigen Positivity

An average of 4.3 days transpires between the beginning of viral shedding and peak viral load with the alpha variant (B1.1.7).⁹² In contrast, the time from exposure to detection was reduced to 3 days with the Delta variant compared with 6 days for the wild-type strain.⁹³ Whether the shortened time it takes for variants to reach peak viral loads warrants a reconsideration of the frequency of serial RADT testing is currently unknown.

Vaccine Breakthrough Cases

Though vaccination reduces the incidence of both asymptomatic and asymptomatic infection even against variants,⁹⁴ breakthrough cases with the Delta variant in vaccinated patients had initial Ct values that were indistinguishable from those occurring in unvaccinated individuals.⁹⁵ The duration of antigen positivity in vaccinated individuals has not been well defined; however, in a study with HCWs, culturable virus was less likely to be recovered from vaccinated HCWs with breakthrough Delta variant infection compared with unvaccinated infected HCWs at 68.6% versus 84.9%, respectively.⁹⁶ Whether antigen positivity mirrors culture positivity among vaccinated individuals with breakthrough infection remains to be determined.

Public Health Reporting

There is generally a lack of structured or automated result reporting to public health authorities for surveillance for many RADTs. In a Kaiser Health News survey, nearly

half of US states indicated antigen test results are underreported.⁹⁷ This can lead to missed opportunities for contact tracing and inaccuracies in tracking positivity rates and potential outbreaks. Furthermore, laboratories are unable to easily follow-up on positive samples for sequencing. This may hamper efforts to detect and track potential variants of concern as they emerge.⁹⁸

Parting Thoughts

The promise of antigen testing is fundamentally associated with its ability to democratize testing, enabling rapid detection of both asymptomatic and symptomatic infection alike and breakage of transmission chains. Nevertheless, the narrative of using RADTs to know one's "infectious status" does not reflect the nuance of testing, that is, that such a status is not static and that transmission among individuals with low viral loads occurs. It is when RADT positivity and contagiousness match that they can have the greatest impact; however, it is often the case that there is a mismatch between the two .

Although it has been proposed that RADTs be considered "public health tests" that are separate from their role as clinical diagnostics, they will invariably be used by symptomatic individuals for personal decision making because results have implications on the health of the test recipient. What has been fundamentally absent in the United States is concerted government-led education around how RADTs should be used and how results should be interpreted along with linkage to confirmatory and timely PCR testing. Unlocking the true power of this technology requires sufficient and cheap/free test supply and sufficient understanding among the general public of its limitations. This may be changing with the free distribution by the US government of RADTs to individuals who request them using an online portal.⁹⁹ As we have highlighted in this review, a multitude of data show that antigen testing works best when used as one element of a coordinated strategy involving vaccination and nonpharmacologic interventions, and not as the sole intervention. Indeed, there are no data published on the impact of frequent RADT surveillance in the absence of other public health measures.

Ultimately, although the promise of antigen testing for SARS-CoV-2 comes from their massive scale, it has been frequently conveyed as if its power comes from individual decision-making. If the true potential of antigen testing is to be harnessed, society would do well to remember the original motto of the United States proposed by the founding fathers: "*E pluribus unum*," or "*out of many, one*." There is no singular solution to ending this pandemic, but certainly, the true power of antigen technology will come from its combination with other public health measures.

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