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PERSPECTIVES

Considerations for Assessment and Deployment of Rapid Antigen Tests for Diagnosis of Coronavirus Disease 2019

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Diagnostic testing is a critical tool to mitigate the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, but molecular testing capacity remains limited. Rapid diagnostic tests (RDTs) that detect SARS-CoV-2 protein antigens (Ag) offer the potential to substantially expand testing capacity and to allow frequent, large-scale, population screening. Testing is simple, rapid (results generally available within 15 minutes), and applicable for diagnosis at point of care. However, implementation of Ag RDTs requires a detailed understanding of test performance and operational characteristics in each testing scenario and population being evaluated. Successful implementation of Ag RDTs on a large scale should combine testing with technical oversight and with clinical and public health infrastructure, and will require production at levels much higher than presently possible. In this commentary, we provide detailed considerations for Ag RDT assessment and use cases to encourage and enable broader manufacturing and deployment.

Keywords. antigen; COVID; diagnostic; point-of-care; SARS-CoV-2.

Determining optimal testing strategies across the diverse populations, communities, and environments impacted by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is complex, and no single testing strategy will address all scenarios. In particular, significant uncertainty exists within the medical community and among the general public regarding optimal use of rapid diagnostic tests (RDTs) that detect SARS-CoV-2 protein antigens (Ags) for diagnosis of coronavirus disease 2019 (COVID-19). We suggest that Ag RDTs with strong analytical and operational performance, when thoughtfully deployed, offer substantial opportunity for mitigation of the COVID-19 pandemic. However, at present, Ag RDTs are not available in sufficient quantities to enable large-scale testing strategies with high potential value for pandemic control. In this study, we provide considerations regarding Ag RDT assessment and use, with the goal of motivating broader manufacturing and deployment.

It is important to recognize that not all SARS-CoV-2 antigen tests demonstrate equivalent performance. Antigen tests with US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) have a broad range of performance characteristics and formats, ranging from simple, visually interpreted Ag RDTs designed for point-of-care (POC) testing to automated immunoassays intended for central laboratories [1, 2]. We caution against generalizations about the performance and use cases for "antigen assays" and instead suggest considering options for deployment of each antigen test separately, based on specific performance data for each test. Each Ag RDT must be studied directly to define its sensitivity, specificity, and operational characteristics, thus allowing informed decisions about feasible use cases.

Concerns surrounding the specificity of Ag RDTs emerged early in the pandemic, leading to significant controversy regarding accuracy. Early reports of unexpectedly high rates of false-positive results were from deployment of Ag RDTs with electronic readers that were first to market [3–5]. In contrast, several tests that are visually read (and thus potentially easier to scale) have demonstrated consistently high specificity (>99%) in field testing [6–12]. Nonetheless, even tests with very high specificity are associated with low positive predictive values when used in populations with low disease prevalence and low pretest probability (eg, asymptomatic individuals with no known exposures to SARS-CoV-2-infected individuals) [13], requiring careful consideration when designing programs for large-scale deployment. As an example, when testing a million individuals with a disease prevalence of .2%,

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even a high-sensitivity test with 99.9% specificity (regardless of format) is expected to be associated with almost as many false-positive results (~1000) as true positives (~2000).

It is challenging to utilize analytical sensitivity data from package inserts to predict the clinical sensitivity of Ag RDTs, in part because limit of detection (LOD) studies have used a variety of methodological approaches and reference materials [1]. In general, Ag RDTs are less sensitive than nucleic acid amplification testing (NAAT). Independent studies comparing the analytical sensitivity of Ag RDTs to NAAT have yielded a wide range of LOD estimates [8, 9, 14]. The best data for Ag RDT performance comes from field study of an Ag RDT at POC in the settings, conditions, and populations of intended use (if available), with manufacturer instructions for use data as a useful baseline by which to compare tests.

The clinical sensitivity of an Ag RDT depends on the distribution of viral loads in the population being tested. Antigen RDTs have high sensitivity when the patient's viral load is high (often defined by the surrogate measure of a low cycle threshold [Ct] value of the amplified target in real-time polymerase chain reaction [PCR] assays). The distribution of viral loads in clinical subgroups of patients differs, and these differences directly impact Ag RDT use cases. Viral loads in adults within the first 7 days of symptom onset seem to be reliably high enough to be able to consistently detect disease in most individuals with the best-performing Ag RDTs [6–8, 11, 14]. However, there has been variability in sensitivity estimates from field studies of individual Ag RDTs in this population (eg, [8, 10, 12]), and the Centers for Disease Control and Prevention (CDC) currently recommends confirming a negative Ag RDT result with an NAAT in this population [2]. The sources of the observed within-test variability in field performance are unclear and require urgent exploration; possibilities include analytical (sample type and NAAT method used as the comparator) and operational (storage, temperature, operator) factors. For diagnostic testing within the first 7 days of symptoms, 2 studies [8, 11] have suggested that Ag RDTs might be slightly less sensitive in children than in adults, and additional studies in symptomatic children are needed. Distribution of viral loads in asymptomatic adults and children detected by one-time screening testing (eg, a drive-through testing site or preprocedure screening) has the widest span, with many individuals having viral loads low enough that they are not or likely would not be detected by Ag RDTs [8, 12, 15]. However, with use of weekly, twice weekly, or more frequent screening, newly infected asymptomatic individuals might have higher viral loads at the time of testing, increasing the clinical sensitivity of Ag RDTs for that use case. Therefore, for large-scale public health surveillance programs in which serial testing could be performed frequently in asymptomatic individuals, the sensitivity of Ag RDTs (if paired with very high specificity) could be sufficient for screening purposes.

Many field studies have used Ct value cutoffs of 25, 30, and 35 (for PCR results from a separate swab collected in parallel) to evaluate Ag RDT sensitivity and reported that Ag RDTs have performed well with Ct cutoffs of ≤25, and in some cases ≤30 [6–11, 16]. The substantial variation in correlation between Ct value and viral ribonucleic acid load in copies/mL between PCR assays [17] is a major caveat to this mode of assessing Ag RDT sensitivity. Nonetheless, these data effectively reiterate that Ag RDTs work best in patients with higher viral load (lower Ct value). Other studies have begun to correlate the ability to culture virus from a clinical sample with the ability to detect infection in that individual with an Ag RDT (eg, [7, 11, 12, 18]). Although there is great variability in the yield of viral culture methods, the data in aggregate suggest that the evaluated Ag RDTs can generally detect most, but not all, individuals whose samples yielded a culturable virus. However, a definitive connection between the inability to culture virus and the inability of that host to transmit to another individual has not been made. Furthermore, it is problematic to assume that individuals with a low viral load (and consequent false-negative Ag RDT result) cannot transmit virus to others because individuals can have low viral loads for more than one reason: they could be very early in infection, have had inadequate specimen collection, or be late in infection (the first 2 scenarios could benefit from repeating the Ag RDT on a subsequent day). Despite these caveats, it is reasonable to assume that individuals with higher viral loads are more likely to transmit to others, and that rapid POC testing and early isolation of Ag-positive individuals will help mitigate the spread of disease.

Much of the controversy about Ag RDT deployment in the COVID-19 pandemic has stemmed from discussions of tests that have remained frustratingly hypothetical: inexpensive, mass-produced tests that could be used frequently for self-testing at home. Although efforts by test manufacturers are moving this idea closer to reality, to date there are only 2 Ag RDTs with EUA for at-home self-testing [1], and neither is inexpensive enough to allow frequent use, particularly in communities with highest need for access to diagnostic and screening testing. This being said, encouraging operational data support the use of Ag RDTs with strong performance in the home environment. One head-to-head study of an Ag RDT showed that sensitivity with self-collected nasal mid-turbinate swabs versus professionally collected nasopharyngeal swab samples was similar [19], and another showed that patients without prior training could successfully follow instructions and perform their own Ag RDTs, obtaining the same results as trained professionals [20] (although the majority of those patients were highly educated). However, other data indicate that reliable visual interpretation of tests performed at home

may not be straightforward: some studies have suggested that specific training in reading positive Ag RDT results is needed to achieve high specificity [9, 10], and others have suggested that the level of training of the operator impacts Ag RDT clinical sensitivity [16]. Finally, reliable self-reporting, quarantine, and other decision-making based on positive and negative test results obtained at home should not be simply assumed. Technologies that automatically read and send Ag RDT results obtained at home to public health systems are a potential solution, but they add expense and complexity. Moreover, the economic and social impacts of positive test results (eg, lost wages) must be anticipated and mitigated.

Given these complexities, we suggest that large-scale public health implementation of Ag RDTs for frequent home use could be done programmatically and with oversight, including a quality assurance program, simple and clear education of the public about test use and results interpretation (highlighting the risk of false negative results), and connection to care, financial assistance, and contact tracing for those with positive results. Such a program could include basic training of each home operator (in person or over video) for first use, clear instructional infographics included in the test kit, and/or mandatory discussion with a care provider at the time of test distribution. Health department (local, state, or CDC) infomercials on TV, radio, and social media could reinforce messaging about results interpretation, self-reporting, and steps to take depending on results. Mass deployment would require substantial manufacturing and implementation resources that to date have not been available, but new investment in this area may be imminent [21].

In parallel with the momentum towards home-based Ag RDT use, we endorse the immediate deployment of Ag RDTs with strong performance (eg, >95% sensitivity in individuals with high viral loads, and >99% specificity) and EUA in large-scale, well organized, easily-accessed testing programs in the hardest-hit communities. Field studies have begun to demonstrate the feasibility and impact of approaches that programmatically combine community-based Ag RDT testing using trained operators and rapid connection to support services and contact tracing for individuals identified as infected [10]. With this systematic deployment strategy, only those performing the test need learn how to do it, no additional FDA approvals are needed, and positive test results can be immediately managed by public health authorities. Individuals performing the tests would have full understanding of the performance and limitations of the test being used and would be in a position to educate those being tested. Large-scale POC Ag RDT deployment using trained operators would immediately facilitate expanded access and ensure optimal sample collection, informed results interpretation and/or reporting, quality control, patient education, and linkage to support services and clinical care. Although Ag RDTs detecting viral nucleoprotein are expected to detect variant SARS-CoV-2 viruses with mutations in the spike protein-encoding gene (eg, UK [B.1.1.7] and South African [B.1.351] variants), the impact of variants on Ag RDT sensitivity can and should be carefully monitored. We further endorse immediate efforts towards mass production of Ag RDTs with demonstrated best performance to allow cost reduction and wide-scale, thoughtful deployment.

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