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Performance of Rapid Antigen Tests to Detect Symptomatic and Asymptomatic SARS-CoV-2 Infection

FREE

A Prospective Cohort Study

Apurv Soni, MD, PhDCarly Herbert, BAHonghuang Lin, PhDYi Yan, PhD

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Annals Author Insight Video - Apurv Soni, MD, PhD, and Carly Herbert, BA

In this video, Apurv Soni, MD, PhD, and Carly Herbert, BA, offer additional insight into the article, "Performance of Rapid Antigen Tests to Detect Symptomatic and Asymptomatic SARS-CoV-2 Infection: A Prospective Cohort Study." (Duration 3:23)

Visual Abstract. Performance of Rapid Antigen Tests to Detect Symptomatic and Asymptomatic SARS-CoV-2 Infection

This prospective study evaluated the performance of rapid antigen tests for detection of SARS-CoV-2 among symptomatic and asymptomatic participants. Participants who were asymptomatic and negative for SARS-CoV-2 on study day 1 completed rapid antigen tests and RT-PCR testing for SARS-CoV-2 every 48 hours for 15 days.

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Abstract

Background:

The performance of rapid antigen tests (Ag-RDTs) for screening asymptomatic and symptomatic persons for SARS-CoV-2 is not well established.

Objective:

To evaluate the performance of Ag-RDTs for detection of SARS-CoV-2 among symptomatic and asymptomatic participants.

Design:

This prospective cohort study enrolled participants between October 2021 and January 2022. Participants completed Ag-RDTs and reverse transcriptase

polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 every 48 hours for 15 days.

Setting:

Participants were enrolled digitally throughout the mainland United States. They self-collected anterior nasal swabs for Ag-RDTs and RT-PCR testing. Nasal swabs for RT-PCR were shipped to a central laboratory, whereas Ag-RDTs were done at home.

Participants:

Of 7361 participants in the study, 5353 who were asymptomatic and negative for SARS-CoV-2 on study day 1 were eligible. In total, 154 participants had at least 1 positive RT-PCR result.

Measurements:

The sensitivity of Ag-RDTs was measured on the basis of testing once (same-day), twice (after 48 hours), and thrice (after a total of 96 hours). The analysis was repeated for different days past index PCR positivity (DPIPPs) to approximate real-world scenarios where testing initiation may not alw coincide with DPIPP 0. Results were stratified by symptom status.

Results:

Among 154 participants who tested positive for SARS-CoV-2, 97 were asymptomatic and 57 had symptoms at infection onset. Serial testing with Ag-RDTs twice 48 hours apart resulted in an aggregated sensitivity of 93.4% (95% CI, 90.4% to 95.9%) among symptomatic participants on DPIPPs 0 to 6.

When singleton positive results were excluded, the aggregated sensitivity on DPIPPs 0 to 6 for 2-time serial testing among asymptomatic participants was lower at 62.7% (CI, 57.0% to 70.5%), but it improved to 79.0% (CI, 70.1% to 87.4%) with testing 3 times at 48-hour intervals.

Limitation:

Participants tested every 48 hours; therefore, these data cannot support conclusions about serial testing intervals shorter than 48 hours.

Conclusion:

The performance of Ag-RDTs was optimized when asymptomatic participants tested 3 times at 48-hour intervals and when symptomatic participants tested 2 times separated by 48 hours.

Primary Funding Source:

National Institutes of Health RADx Tech program.

Diagnostic testing for SARS-CoV-2 remains a cornerstone in our nation's fight against COVID-19, and at-home rapid antigen tests (Ag-RDTs), although not perfect, provide a fast and convenient testing option. This type of test is available without a prescription (that is, over-the-counter [OTC]), is easy to use, is widely available, and in some cases is preferred by the population over molecular assays that require appointments, waiting in line at testing centers, and waiting 24 to 48 hours for results (1–3). Despite the popularity of Ag-RDTs, key gaps remain in our understanding of these tests, notably their performance as a screening tool among asymptomatic people. Reports on

Ag-RDT performance among persons testing while asymptomatic have been highly varied, with sensitivities ranging from 35.8% to 71% in cross-sectional screening evaluations (4, 5). However, performance has typically been evaluated on the basis of the single use of an Ag-RDT, and few studies have evaluated serial testing performance of Ag-RDTs among asymptomatic persons. Furthermore, emergency use authorization (EUA) of OTC antigen tests by the U.S. Food and Drug Administration (FDA) required a postauthorization demonstration of Ag-RDT performance in a population with asymptomatic infection using serial testing. This article describes primary findings from a large study designed in coordination with the National Institutes of Health, FDA, and 3 major Ag-RDT manufacturers to evaluate the performance of serial testing using Ag-RDTs for detection of SARS-CoV-2 among asymptomatic persons within the first week of infection. A primary goal of this study was to provide broadly applicable data that could be leveraged to satisfy the postauthorization requirement for all authorized OTC antigen tests.

Methods

Study Population and Design

Between 18 October 2021 and 31 January 2022, this 15-day prospective cohort study enrolled participants older than 2 years from across the country through a novel, digital, siteless study protocol. This study was approved by WIRB-Copernicus Group Institutional Review Board (20214875). Participants were eligible to enroll through a smartphone app if they had not had a SARS-

CoV-2 infection in the prior 3 months, had been without any symptoms in the 14 days before enrollment, and were able to drop off prepaid envelopes with nasal swab samples at their local FedEx drop-off location. The smartphone app was freely available, and the study was advertised by local public health departments across the United States. Enrolled participants were assigned to 1 of 3 types of Ag-RDT with EUA (Quidel QuickVue At-Home OTC COVID-19 Test, BinaxNOW COVID-19 Antigen Self Test, or BD Veritor At-Home COVID-19 Test) on the basis of an automated algorithm based on enrollment numbers and geographic location of the participants. They received a home delivery of 10 Ag-RDTs and 7 home collection kits for reverse transcriptase polymerase chain reaction (RT-PCR) samples. Participants were asked to perform 2 self-collected bilateral anterior nasal swab collections and paired testing (Ag-RDT [at home] and RT-PCR [mailed to central laboratory]) between study day 1 and study day 13 on 48-hour intervals, with an additional end-of-study bilateral anterior nasal swab collection for a home Ag-RDT on study day 15. Two FDA-authorized, high-sensitivity RT-PCR assays were done on each nasal swab sample received at the central laboratory, and an additional tiebreaker assay was done if [illegible] RT-PCR assays were discordant. Additional details about the study des[illegible] recruitment, and protocol are described elsewhere (6).

Measures

Results of Ag-RDTs were based on self-reporting (Quidel QuickVue At-Home OTC COVID-19 Test and BinaxNOW COVID-19 Antigen Self Test) or an automatic reader (BD Veritor At-Home COVID-19 Test), according to the EUA

instructions for use. Molecular comparator RT-PCR results were based on a combination of molecular test results for detection of SARS-CoV-2 infection (Supplement Table 1), and onset of infection was defined as the day on which the molecular comparator result was positive for the first time. Cycle threshold (Ct) values for the *E* gene from 1 RT-PCR test were used as a measure to quantify viral load. To approximate the performance of Ag-RDTs if a person started testing on different days from onset of infection, we identified days past index PCR positivity (DPIPPs) as different strata, for which we calculated performance. Symptomatic or asymptomatic classification was based on the presence or absence of symptoms on the DPIPP for which the performance was calculated. Therefore, a person who was asymptomatic on DPIPP 0 may have become symptomatic on DPIPP 2 and vice versa.

Statistical Analysis

Participants were eligible for inclusion in this analysis if they did not report any symptoms and had molecular tests and Ag-RDTs negative for SARS-CoV-2 on study day 1. We decided to pool performance across the tests becau findings not shown in this report suggested that various Ag-RDTs have similar sensitivity to each other as a function of the viral load and because the study was not designed to evaluate differences in performance among the 3 types of Ag-RDTs. Performance was calculated using sensitivity (rapid antigen positivity / comparator positivity) for single-day testing, 2-time serial testing at 48-hour intervals, and 3-time serial testing at 48-hour intervals for symptomatic and asymptomatic persons based on day and patterns of

positivity, as described in Supplement Table 2. Calculations for sensitivity were repeated with testing starting on different DPIPPs. We estimated CIs for 1-week sensitivity using the bootstrapping technique (7). Specificity was calculated as a proportion of paired tests (Ag-RDT and molecular test done on the same day) based on the following formula: true negative / [false positive + true negative], where true negative refers to a molecular test with negative results paired with an Ag-RDT with negative results on the same day and false positive refers to a molecular test with negative results paired with an Ag-RDT with positive results. To evaluate the viral dynamics during infection, we compared mean Ct values and 95% CIs between symptomatic and asymptomatic participants by DPIPP. A mixed-effects logistic regression model was used to predict the probability of Ag-RDT positivity based on Ct value and symptom status. Cycle threshold values, symptom status, rapid antigen testing series (1, 2, or 3 tests), and their 3-way interactions were fixed effects, whereas participant identification number was included as a random effect. All analyses were done using R, version 4.1.1 (R Foundation) (8).

Role of the Funding Source

The National Institutes of Health had no role in the study design; collection, analysis, or interpretation of data; writing of the report; or decision to submit the manuscript for publication.

Results

Performance of Serial Testing With Ag-RDTs Among Symptomatic and Asymptomatic Participants

A total of 7361 participants enrolled in the study, and 5353 were eligible for this analysis, with a total of 42 130 days of testing. Participant age ranged from 2 to 90 years, with a mean of 37.4 years (SD, 17.0). In total, 1817 (33.9%), 1805 (33.7%), and 1731 (32.3%) eligible participants were assigned to Quidel QuickVue At-Home OTC COVID-19 Test, BinaxNOW COVID-19 Antigen Self Test, and BD Veritor At-Home COVID-19 Test, respectively. Approximately 13.3% of participants were unvaccinated for SARS-CoV-2, 4.9% had received 1 dose of a SARS-CoV-2 vaccine, and 81.7% had received 2 or more doses of a SARS-CoV-2 vaccine. In total, 16 (3.7%) Ag-RDTs with positive results and 1948 (5.6%) with negative results were missing a scheduled, corresponding, paired RT-PCR test, and these data points were excluded in the analysis. Of the 34 737 RT-PCR tests with negative results, 1182 (3.4%) were missing a corresponding Ag-RDT result. Just 7 (1.5%) RT-PCR tests with positive results were missing an Ag-RDT result from the same day. Of the participants eligible for analysis, 154 tested positive for SARS-CoV-2 on RT-PCR during the study on the basis of a composite definition described in Supplement 1; of these, 97 were without symptoms and 57 had symptoms at infection onset (Figure 1). Among the 5199 participants who did not test positive, there were 32 998 days of paired Ag-RDT and RT-PCR testing where the comparator result was negative. Among these, 32 862 days had a concordant Ag-RDT negative result, yielding a specificity of 99.6% (95% CI, 99.5% to 99.7%).

Figure 1. Study flow diagram.

In the Test Us At Home study to calculate performance of Ag-RDTs for detection of SARS-CoV-2, a total of 7361 participants enrolled in the study, and 154 were eligible for the analysis and tested positive for SARS-CoV-2 by RT-PCR during the study period (97 asymptomatic and 57 symptomatic on day of index comparator positive result). Ag-RDT = rapid antigen test; OTC = over-the-counter; RT-PCR = reverse transcriptase polymerase chain reaction. * Participants replaced their assigned Ag-RDTs with commercially obtained Ag-RDTs. † Dates of RT-PCR testing could not be verified based on triangulation of self-reported, shipping, and resulting data.

The performance of Ag-RDTs to detect SARS-CoV-2 on the day of infection onset (DPIPP 0) was higher among symptomatic participants (59.6% [CI, 46.7% to 71.4%]) than asymptomatic participants, where fewer than 10% of infections were detected by Ag-RDT on DPIPP 0 (9.3% [CI, 5.0% to 16.7%]) (Figure 2; Supplement Table 3). Performance improved with serial testing with 2 Ag-RDTs 48 hours apart (symptomatic: 92.2% [CI, 81.5% to 96.9%]; asymptomatic: 39.3% [CI, 29.8% to 49.7%]) and 3 Ag-RDTs 48 hours apart (symptomatic: 93.6% [CI, 82.8% to 97.8%]; asymptomatic: 56.4% [CI, 45.4% to 66.9%]).

Figure 2. Performance of Ag-RDTs for detection of SARS-CoV-2 in relation to first day of mole positivity.

Sensitivity (rapid antigen positivity / comparator positivity) was calculated for single-day testing, 2-time serial testing at 48-h intervals, and 3-time serial testing at 48-h intervals for symptomatic and asymptomatic persons based on day and patterns of positivity. Calculations for sensitivity were repeated with testing starting on different days past index positivity on an RT-PCR test. Error bars represent 95% CIs. Performance of Ag-RDTs on day of infection onset was higher among symptomatic participants (59.6%) than among asymptomatic participants (9.3%). Serial testing with 2 Ag-RDTs 48 h apart and 3 Ag-RDTs 48 h apart improved the performance of Ag-RDTs within the first week of infection. Excluding participants with singleton RT-PCR–positive results improved the sensitivity of Ag-RDTs among asymptomatic participants. Ag-RDT = rapid antigen test; DPIPP = day past index polymerase chain reaction positivity; RT-PCR = reverse transcriptase polymerase chain reaction.

Of note, 20 participants had a singleton positive result on RT-PCR, defined as a test with a positive result preceded and followed by an RT-PCR test with a negative result within 48 to 54 hours. Of those with singleton RT-PCR positivity, none tested positive on an Ag-RDT, only 1 had symptoms on the day of singleton positivity, and the average Ct value was above 35 (Supplement Figure). Excluding these participants did not affect the sensitivity of Ag-RDTs among symptomatic participants, but it improved asymptomatic sensitivity to 11.7%, 50.7%, and 74.6% based on testing 1, 2, and 3 times, respectively, with Ag-RDTs at 48-hour intervals beginning on DPIPP 0.

Performance of Ag-RDTs by DPIPP

To approximate real-world scenarios, where a person may not necessarily start testing with Ag-RDTs on the day of infection onset, we calculated performance separately on DPIPPs 2, 4, 6, 8, and 10 (Figure 2; Supplement Table 3) to approximate scenarios where a person started serially testi with Ag-RDTs on those days. The aggregated performance of Ag-RDTs DPIPPs 0 to 6 among all participants who were symptomatic on a given DPIPP was 82.5% (CI, 78.3% to 86.3%) for single-time-point testing, but sensitivity (calculated on a per DPIPP basis) ranged from 59.6% to 94.8%. Serial testing improved sensitivity to 93.4% (CI, 90.4% to 95.9%) using 2-time testing and 94.3% (CI, 91.4% to 97.0%) using 3-time testing. The sensitivity of a single test, 2-time serial testing, and 3-time serial testing for asymptomatic

people was 34.4% (CI, 28.8% to 39.8%), 55.3% (CI, 48.2% to 61.6%), and 68.5% (CI, 61.0% to 75.7%), respectively, during the first week of infection (DPIPPs 0 to 6). When singleton positive results on RT-PCR were excluded, the first-week (DPIPPs 0 to 6) sensitivity for asymptomatic persons was 38.8% (CI, 32.7% to 45.2%), 62.7% (CI, 57.0% to 70.5%), and 79.0% (CI, 70.1% to 87.4%) for testing 1, 2, and 3 times, respectively, with Ag-RDTs at 48-hour intervals.

Ct Values Among Symptomatic and Asymptomatic Participants

The performance of Ag-RDTs among symptomatic and asymptomatic participants was evaluated by Ct value to analyze the performance by viral load. Among RT-PCR tests with positive results, 17 (4.0%) were missing Ct values and were excluded from Ct value analyses. The distribution of Ct values significantly differed between symptomatic and asymptomatic participants, with symptomatic participants having lower Ct values on average than asymptomatic participants at DPIPPs 0 and 2 (Figure 3). On the day of index PCR positivity, more than 75% of asymptomatic persons had a Ct value of 30 or higher, whereas fewer than 33% of symptomatic persons had a Ct value of 30 or higher. At the end of 1 week from index PCR po (DPIPP 6), the majority of both asymptomatic and symptomatic persons had a Ct value lower than 30. Figure 4 shows the sensitivity of Ag-RDTs for different Ct values based on postestimation results from a multilevel model. The sensitivity was lowest among asymptomatic participants who did a single test for all Ct values greater than 20, compared with symptomatic participants who did a single test and symptomatic and asymptomatic participants who did 2-time and 3-time serial testing (Figure 4; Supplement

Table 4). Two-time serial testing among symptomatic persons and 3-time serial testing among both asymptomatic and symptomatic persons had a sensitivity above 80% for Ct values lower than 32.

Figure 3. Ct values, by DPIPP and symptom status.

Error bars represent 95% CIs. Symptomatic participants who tested positive by reverse transcriptase polymerase chain reaction (RT-PCR) had significantly lower Ct values on average than asymptomatic participants at DPIPPs 0 and 2. On DPIPP 0, >75% of asymptomatic persons had a Ct value ≥30, whereas <33% of symptomatic persons had a Ct value ≥30. Symptomatic participants had a lower proportion of persons with Ct values ≥30 at all DPIPPs. Ct = cycle threshold; DPIPP = day past index polymerase chain reaction positivity.

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Figure 4. Predicted probability of Ag-RDT positivity, by symptom status and serial testing schedule.

A mixed-effects logistic regression model was used to predict the probability of Ag-RDT positivity based on Ct value and symptom status. Error bars represent 95% CIs. The sensitivity was lowest among asymptomatic participants who performed a single test for all Ct values >20. Two-time serial testing among symptomatic persons and 3-time serial testing among both asymptomatic and symptomatic persons demonstrated sensitivity >80% for Ct values <32. Ag-RDT = rapid antigen test; Ct = cycle threshold; RT-PCR = reverse transcriptase polymerase chain reaction.

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Discussion

Here we report findings from the largest study to date of paired Ag-RDT and RT-PCR testing for a comparative performance evaluation of Ag-RDTs among community-dwelling children and adults with and without symptoms. These results strongly suggest that Ag-RDT testing should include additional,

repeated testing. We found an improvement in test performance when symptomatic persons tested 2 times, 48 hours apart, using Ag-RDTs. Likewise, performance improved further in asymptomatic persons when an initial Ag-RDT was followed by at least 2 subsequent tests at 48-hour intervals. These results should be considered in the context of our study protocol, which indicated testing at 48-hour intervals; thus, these data cannot support conclusions about serial testing for time intervals shorter than 48 hours.

These findings represent a comprehensive evaluation of the time-dependent performance of Ag-RDTs among the intended use population (that is, symptomatic and asymptomatic persons) throughout the course of molecular test positivity. Restricting findings from our study to match observation windows from previous studies, we found sensitivity similar to that found in previous studies for asymptomatic and symptomatic participants for a single-time test (9). Unlike previous reports, which used composite sampling methods and lacked sufficient longitudinal data to adequately evaluate the performance of Ag-RDTs from the onset of infection, we could approximate the performance of Ag-RDTs for symptomatic a asymptomatic users by comparing performance within the first week of infection, to align with the indications listed in the EUA, and to evaluate the performance of serial testing within this paradigm (10, 11).

Of note, more than 1 in 10 new SARS-CoV-2 infections was a singleton positive result and escaped detection by Ag-RDTs. Evaluation of singleton positive infections showed that all of them had Ct values above 30, with an

average Ct count of 35. This finding needs to be further investigated to understand the clinical significance of singleton positive infections.

Our finding of higher Ct values associated with lower sensitivity is in line with results from a comprehensive meta-analysis encompassing data from 214 clinical studies and 112 323 samples, which demonstrated that the sensitivity of rapid antigen testing deteriorated with increasing Ct values (9). We also observed that Ag-RDTs have higher sensitivity among symptomatic participants, regardless of Ct value. The finding that the performance of Ag-RDTs differed with respect to Ct values between symptomatic and asymptomatic participants was unexpected because Ag-RDT performance has often been considered to be a function of viral load (12, 13); however, our finding may suggest that viral dynamics are not the only factor. Symptomatic and asymptomatic persons may differ in at least 3 other domains: interpretation of results, administration of tests, and physiology (that is, amount of secretions available for sampling). Previous work found that symptom status was not a predictor of false-negative results; however, self-interpretation of results may introduce bias because people's own understanding of their COVID-19 risk may influence their level of cau when interpreting tests (14). These 3 hypotheses are subject to further inquiry because it is important to determine the role of these factors in Ag-RDT performance. In addition, a previous report suggested that people with certain haplotypes of the HLA loci are 2 to 8 times more likely to have an asymptomatic infection, and in a large, genome-wide association study, this haplotype was found to be prevalent in roughly 10% of patients (15). Similar observations have been made with HIV, where certain genotypes are

associated with lower propensity of infection. The effect of HLA haplotype on SARS-CoV-2 infections should be investigated further.

This study does have limitations. It was done during the circulation of the Delta and Omicron SARS-CoV-2 variants, and future variants may warrant further investigation, especially as milder, less symptomatic variants emerge (16, 17). In addition, specimens were self-collected for RT-PCR, and Ag-RDTs were self-performed. However, data have consistently shown substantial agreement between self-collected and clinician-collected anterior nasal swabs for SARS-CoV-2 testing (18, 19). Further, this primary analysis does not account for differences in severity or type of symptoms.

The public health implications of our findings are that people testing for SARS-CoV-2 should exercise caution despite an initial negative result on an Ag-RDT and favor mask wearing and avoiding crowded places if they suspect they may be infected or have been exposed. In addition, the rates of false-positive results in the study were low; therefore, any Ag-RDT–positive result should be considered positive without the need to retest. Further, in the context of reports of viral culture positivity more than 5 days after an i positive result, our findings support isolation for a longer period to pr the potential spread of SARS-CoV-2 to others (20). Further research is needed to quantitatively estimate the benefits of Ag-RDTs for early detection of infection and initiation of treatment, especially in settings where access to molecular testing is limited or molecular test results are delayed. Dissemination of clear guidance for appropriate testing using Ag-RDTs based on data from this study may help preserve confidence in the performance of

serial Ag-RDTs to detect SARS-CoV-2, especially as reports of individual false-negative Ag-RDT results from inadequate serial testing, contrary to the tests' intended usage and guidance from the FDA, proliferate in lay media.

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