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Comparative diagnostic utility of SARS-cov-2 rapid antigen and molecular testing in a community setting

Ashley E. Kim, MPH[1,2]; Julia C. Bennett, MSPH[1,3]; Kyle Luiten, BS[1]; Jessica A. O'Hanlon, BS[1]; Caitlin R. Wolf, BS[1]; Ariana Magedson, BSW[1]; Peter D. Han, MS[4,5]; Zack Acker, MSCM[4,5]; Lani Regelbrugge, BS[4,5]; Kathryn M. McCaffrey, BS[4]; Jeremey Stone, BA[4,5]; David Reinhart, BA[4,5]; Benjamin J. Capodanno, BS[4,5]; Stephen S. Morse, PhD[2]; Trevor Bedford, PhD[4,5,6,9]; Janet A. Englund, MD[10]; Michael Boeckh, MD PhD[1,6]; Lea M. Starita, PhD[4,5]; Timothy M. Uyeki, MD MPH[7]; Marco Carone, PhD[8]; Ana Weil, MD MPH[1]; Helen Y. Chu, MD MPH[1]

[1] Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, WA, USA [2] Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, USA [3] Department of Epidemiology, University of Washington, Seattle, WA, USA [4] Brotman Baty Institute for Precision Medicine, Seattle, WA, USA [5] Department of Genome Sciences, University of Washington, Seattle, WA, USA [6] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Seattle, WA, USA [7] Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA [8] Department of Biostatistics,

Corresponding Author: Ashley E. Kim, MPH, Center for Emerging and Re-emerging Infectious Diseases, 750 Republican Street, UW Medicine Box 358061, Seattle, WA 98109, USA, ashleyek@uw.edu

Alternate Corresponding Author: Helen Y. Chu, MD MPH, Center for Emerging and Re-emerging Infectious Diseases, 750 Republican Street, UW Medicine Box 358061, Seattle, WA 98109, USA, helenchu@uw.edu

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University of Washington, Seattle, WA, USA [9] Howard Hughes Medical Institute, Seattle, WA, USA [10] Seattle Children's Research Institute, Seattle, WA, USA

Background. SARS-CoV-2 antigen-detection rapid diagnostic tests (Ag-RDTs) have become widely utilized but longitudinal characterization of their community-based performance remains incompletely understood.

Methods. This prospective longitudinal study at a large public university in Seattle, WA utilized remote enrollment, online surveys, and self-collected nasal swab specimens to evaluate Ag-RDT performance against real-time reverse transcription polymerase chain reaction (rRT-PCR) in the context of SARS-CoV-2 Omicron. Ag-RDT sensitivity and specificity within 1 day of rRT-PCR were evaluated by symptom status throughout the illness episode and Orf1b cycle threshold (Ct).

Results. From February to December 2022, 5,757 participants reported 17,572 Ag-RDT results and completed 12,674 rRT-PCR tests, of which 995 (7.9%) were rRT-PCR-positive. Overall sensitivity and specificity were 53.0% (95% CI: 49.6 – 56.4%) and 98.8% (98.5 – 99.0%), respectively. Sensitivity was comparatively higher for Ag-RDTs used 1 day after rRT-PCR (69.0%), 4 to 7 days post-symptom onset (70.1%), and Orf1b Ct ≤20 (82.7%). Serial Ag-RDT sensitivity increased with repeat testing ≥2 (68.5%) and ≥4 (75.8%) days after an initial Ag-RDT-negative result.

Conclusion. Ag-RDT performance varied by clinical characteristics and temporal testing patterns. Our findings support recommendations for serial testing following an initial Ag-RDT-negative result, especially among recently symptomatic persons or those at high-risk for SARS-CoV-2 infection.

Keywords: COVID-19, Omicron, self-tests, Ag-RDT, PCR, serial testing, community setting, concordance, sensitivity, symptom status

BACKGROUND

Accessible and reliable diagnostic tests to detect severe respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has remained a public health priority since the onset of the COVID-19 pandemic. Considered the gold standard for diagnosis of COVID-19, the real-time reverse transcription polymerase chain reaction (rRT-PCR) assay is a highly sensitive laboratory-based nucleic-acid amplification assay that detects SARS-CoV-2 infection in both symptomatic and asymptomatic individuals.[1] In 2020, pandemic mitigation guidance from the Centers for Disease Control and Prevention (CDC) included recommendations for rRT-PCR testing following the onset of COVID-like illness (CLI) symptoms, exposure to persons who recently tested positive for SARS-CoV-2, or before and after high-risk activities, such as travel or indoor gatherings.[2] Subsequently, rRT-PCR testing programs were scaled-up globally to meet the unprecedented demand for diagnostic testing. However, despite its high sensitivity, sustained and

frequent use of rRT-PCR testing poses feasibility challenges due to cost and requirements for laboratory space, reagents, and trained personnel.[3]

Antigen-detection rapid diagnostic tests (Ag-RDTs) are a point-of-care self-testing option with advantages including timeliness of results, relative affordability, and convenience compared to rRT-PCR assays. Since the Food and Drug Administration issued its first emergency use authorization for a Ag-RDT in December 2020, at-home diagnostics to detect COVID-19 have become increasingly available and widely used.[4] Compared to rRT-PCR, initial Ag-RDT performance analyses against wild type SARS-CoV-2 or the Alpha and Delta variants yielded sensitivities and specificities ranging from 50 to 80% and 97 to 100%, respectively.[3,5–8] However, it is important to reassess Ag-RDT accuracy as new variants of concern (VOCs) with considerable mutations compared to ancestral SARS-CoV-2 emerge and predominate, such as Omicron.[9] Additionally, longitudinal characterization of Ag-RDT performance over the course of an illness episode in highly-vaccinated, community-based settings remain incompletely understood. This study aims to evaluate Ag-RDT performance with rRT-PCR and identify characteristics associated with reduced diagnostic accuracy in the context of SARS-CoV-2 Omicron predominance.

METHODS

Study design

The Husky Coronavirus Testing Study provided voluntary SARS-CoV-2 testing to students, faculty, and staff at the University of Washington, a large public university in Seattle, Washington, USA. The research study design, data collection, and laboratory methods have been previously described.[10,11] Eligible participants were ≥13 years, had a valid university identification number, lived within a 100-mile radius of the Seattle or two nearby satellite campuses, and provided informed consent in English. Participants self-reported baseline demographic, social, and behavioral information through an electronic questionnaire, including sex, race, ethnicity, on-campus visit frequency, and household characteristics. Additional electronic questionnaires were administered regularly to ascertain updated eligibility and other demographic information. Electronic questionnaires and data management were conducted through REDCap.[12,13]

During the 2021-2022 academic year, participants received a daily attestation survey via email or text message and were invited to self-test following report of out-of-state travel, exposure to a known SARS-CoV-2 case, and new or worsening COVID-19 symptoms (Supplemental Table 1). Additionally, members of campus groups experiencing an outbreak were invited to test, and walk-in testing was available at any time. Self-collected anterior nasal swabs for rRT-PCR testing were supervised when conducted at staffed on-campus testing sites, and unsupervised for samples returned via on-campus drop boxes or by rapid courier. Nasal swab specimens were

tested for SARS-CoV-2 using a laboratory-developed rRT-PCR test at the Brotman Baty Institute at the University of Washington and results were provided to participants through a secure electronic portal.[14] rRT-PCR-positive specimens with a high quantity of SARS-CoV-2 RNA underwent genomic sequencing, as previously described.[10] Beginning late-February 2022, questions were added to the daily attestation survey to collect self-reported SARS-CoV-2 Ag-RDT testing dates and results. Ag-RDTs were acquired through pharmacies, government-supplied programs, or the university (Supplemental Figure 1). University-provided Flowflex Ag-RDTs were free and available to participants at rRT-PCR testing sites. Participants could complete Ag-RDTs at any time and report their results from the past 7 days through electronic daily attestations.

Data analysis

Ag-RDT performance was assessed among participants having undergone ≥1 Ag-RDT within 7 days of rRT-PCR, which was used as the reference standard. We defined SARS-CoV-2 infection as a laboratory-confirmed rRT-PCR-positive result with a cycle threshold (Ct) <40, as previously described.[10,11] For concordance analyses, each Ag-RDT was matched to the closest rRT-PCR by test date within 7 days. Positive and negative concordance were defined as an Ag-RDT result matching a positive or negative rRT-PCR result, respectively. Sensitivity and specificity estimates were calculated among Ag-RDTs performed within 1 day of rRT-PCR, and obtained using intercept-only logistic regression models fitted on the relevant subset of the data using generalized estimating equations (GEE) under an independence working correlation structure.[15] While such estimates agree exactly with empirical sensitivity and specificity estimates, the use of GEEs and robust standard errors facilitated the construction of 95% Wald confidence intervals (CIs) accounting for potential intraparticipant correlation from repeated sampling. For each stratification factor, comparisons of sensitivity and specificity across strata were performed using inverse variance-weighted multivariate Wald tests of the null hypothesis that all non-intercept coefficients are zero in GEE-fitted logistic regression models including only stratum indicators.

All symptom and vaccine status data were self-reported. Symptom status was defined relative to rRT-PCR testing and for the illness episode overall. Participants who reported symptom onset 7 days prior to or on the date of rRT-PCR testing were considered symptomatic. Individuals who reported symptoms presence in the 7 days after rRT-PCR testing were considered asymptomatic at rRT-PCR but symptomatic for the illness episode. A participant was considered fully vaccinated two weeks after completing the primary COVID-19 vaccine series and boosted two weeks after receiving a booster dose for fully vaccinated persons. Participants who received less than a full primary series or reported no prior COVID-19 vaccination at the time of rRT-PCR testing were categorized as unvaccinated, and those who provided invalid vaccination dates or did not report any information were classified as having an unknown vaccination status. Data cleaning and statistical analyses were performed in R and SAS.

RESULTS

Participant characteristics

A total of 5,575 participants who reported an Ag-RDT result within 7 days of rRT-PCR testing from February 23 to December 14, 2022, were included in this analysis (Table 1). Median age was 29 years (range: 18 to 82 years), over half of participants were female (67%), and most were white (60%) or Asian (26%). The sample of study participants included 54.3% students, including 3.6% fraternity and sorority community members, and 44.8% staff and faculty. Most participants were vaccinated against COVID-19 at the time of their first rRT-PCR test during the analysis period, including 78% (N = 4,332) who received a monovalent booster and 14% (N = 800) who received the primary series only. Only 0.7% of participants were unvaccinated and vaccination status was unknown for 7.3%. University policies were updated making indoor masking optional starting March 28, 2022, in alignment with CDC guidance on February 25, 2022.[16,17] Despite relaxed COVID-19 mandates, many participants continued to sometimes or always adhere to non-pharmaceutical interventions throughout the study, with only 0.8% and 4.5% reporting never using a mask or social distancing during the analysis period, respectively.

SARS-cov-2 Results and Clinical Characteristics

A total of 12,674 rRT-PCR and 17,572 Ag-RDT results from 5,575 individuals were included in this analysis. A positive result was reported for 8% (N = 1,350) of the 17,572 Ag-RDTs performed within 7 days of rRT-PCR testing. Of the 12,674 rRT-PCR samples, 995 (8%) were SARS-CoV-2-positive. The results of this analysis should be interpreted in the context of the predominant SARS-CoV-2 lineages circulating during the study period. Genomic sequencing of 584 (59%) rRT-PCR-positive samples from 515 participants identified Omicron BA.2 (N = 277, 47%), BA.5 (N = 134, 23%), and BA.2.12.1 (N = 114, 20%) as the predominant lineages. Among 12,674 rRT-PCRs, 43% (N = 5,440) of participants were symptomatic at testing whereas 5% (N = 694) became symptomatic in the following 7 days resulting in 6,134 (48%) symptomatic illness episodes. Of the 6,134 rRT-PCRs where participants had symptomatic illness episodes, 14% (N = 882) were rRT-PCR-positive, representing 89% of the 995 positive results. Among these 882 symptomatic SARS-CoV-2-positive individuals, the most reported symptoms were sore throat (72%), cough (59%), and rhinorrhea/congestion (55%; Supplemental Table 1). The most predictive self-reported symptoms of rRT-PCR-positivity (number of positive results out of all tests where a symptom was reported within 3 days) were loss of taste or smell (26/62, 42%), chills (208/604, 34%), sweats (137/422, 33%), and feeling feverish (282/923, 31%). Similarly, the most predictive symptoms of Ag-RDT-positivity were loss of smell or taste (38/113, 34%), sweats (176/715, 25%), chills (246/1,018, 24%), and rash (14/60, 23%).

Ag-RDT Performance

Among 7,704 Ag-RDTs performed within 1 day of 860 rRT-PCR-positive and 6,844 rRT-PCR-negative tests from 3,918 individuals, estimated overall sensitivity was 53.0% (49.6 – 56.4%)

and specificity was 98.8% (98.5 – 99.0%; Figure 1). Adjusted for potential intraparticipant correlation, estimated positive predictive value (PPV) was 84.8% (81.4 – 87.6%) and negative predictive value (NPV) was 94.4% (93.7 – 95.0%) for 7.9% SARS-CoV-2 positivity in the study overall. Based on the overall sensitivity (53.0%) and specificity (98.8%) estimates, probability curves were constructed to estimate PPV and NPV for prevalence ranging from 2% to 14% (Figure 2). We also evaluated the probability of detecting SARS-CoV-2 infection with multiple Ag-RDT tests from days -1 to +1 and +7 of a positive rRT-PCR (Supplemental Table 2). The probability of a positive result for at least one out of all Ag-RDT performed within 1 day was 62.5% (58.8 – 66.1%) versus 66.0% (62.3 – 69.3%) for all Ag-RDTs performed through the 7 days after a positive rRT-PCR.

Ag-RDT sensitivity varied by symptom status at rRT-PCR and was higher for symptomatic (53.9%, 50.3 – 57.4%) versus asymptomatic (44.0%, 32.3 – 56.4%) individuals ($p > 0.05$). COVID-like illness was defined as acute onset of at least one symptom of cough, loss of taste or smell, difficulty breathing or chest pain, or at least two symptoms of fever, chills, muscle or aches, headache, sore throat, nausea or vomiting, diarrhea, fatigue, or runny nose within 3 days of RT-PCR testing.[18] Ag-RDTs were 56.3% (52.4 – 60.2%) sensitive among participants who met the CLI definition and 43.9% (37.5 – 50.5%) for those who did not ($p < 0.01$). Among symptomatic participants, sensitivities were significantly different by days from symptom onset to first test date of each Ag-RDT-to-RT-PCR match ($p < 0.001$). Sensitivity was 41.2% (35.3 – 47.4%) on the day of symptom onset and increased to 70.1% (58.1 – 79.9%) for tests used 4 to 7 days after. Conversely, estimated specificity was high for both, but slightly higher among asymptomatic (99.6%, 99.4 – 99.8%) than symptomatic (97.8%, 97.3 – 98.3%) persons ($p < 0.001$).

Estimated Ag-RDT sensitivity and specificity were comparable regardless of COVID-19 vaccination status and supervised versus unsupervised rRT-PCR sample collection (Figure 1; $p > 0.05$). Estimated sensitivity was highest when the Ag-RDT was performed 1 day after a positive RT-PCR (69.0%, 59.9 – 76.9%) versus the same day (62.0%, 57.2 – 66.6%) or 1 day before (38.3%, 33.5 – 43.5%; $p < 0.001$). In contrast, estimated specificity was highest for Ag-RDTs performed 1 day before a negative rRT-PCR (99.5%, 99.1 – 99.7%), compared to 1 day after (95.7%, 94.3 – 96.8%) or the same day (99.3%, 98.9 – 99.5%; $p < 0.001$). The association between Orf1b Ct, analyzed categorically, and sensitivity was assessed among individuals who performed an Ag-RDT within 1 day of a rRT-PCR-positive sample. Lower Orf1b Ct (i.e., higher semiquantitative viral loads) were associated with notably higher estimated sensitivity: 82.7% (72.0 – 89.8%) for Ct ≤20 compared to 36.5% (30.4 – 43.0%) for Ct between 30 to 35 (Figure 1; $p < 0.001$). Mean Ct values were lower for rRT-PCR-positive tests with a concordant Ag-RDT-positive result within 1 day and were lowest among those performed 3 days after symptom onset (24.3, Std: 6.3 cycles; Figure 3).

Among 17,572 Ag-RDTs matched to the closest rRT-PCR test date within 7 days, negative concordance (95.6%, range: 89.9 – 99.0%) was higher than positive concordance (52.9%, range: 36.9 – 73.0%; Figure 4a, Supplemental Table 3). Positive concordance was highest when the Ag-RDT was performed 1 to 7 days after rRT-PCR (69.2%, range: 62.9 – 73.0%), compared to 1 to 7 days before (40.2%, range: 36.9 – 67.7%) or the same day (59.3%). Positive concordance was low for asymptomatic individuals (39.3%, range: 22.0 – 66.7%) and among symptomatic individuals when Ag-RDT was performed 1 to 7 days prior to rRT-PCR (39.9%, range: 36.5 – 68.0 %; Figure 4b-c, Supplemental Table 4).

Serial testing

Serial testing was examined longitudinally over a 15-day period among 756 Ag-RDTs with ≥1 discordant result within 1 day of 177 rRT-PCR-positive tests of which, estimated overall sensitivity was 51.2% (47.7 – 54.7; Figure 5-6). Sensitivity was significantly higher with Ag-RDT use 1 to 7 days after rRT-PCR (61.9%, 55.7 – 67.7%) compared to 1 to 7 days before (38.2%, 32.2 – 44.6%) and same day testing (60.0%, 49.1 – 70.0%; p <0.001). Likewise, sensitivity was significantly different by time from Ag-RDT to symptom onset (p <0.001). Ag-RDTs were least sensitive when used 1 to 7 days before (12.5%. 6.5 – 22.7%) and the same day as symptom onset (26.1%, 18.0 – 36.3%). Sensitivity increased substantially when Ag-RDTS were used 1 to 3 days (62.3%, 53.3 – 70.4%) and 4 to 7 days (82.6%, 75.7 – 87.9%) after symptom onset, but were only 49.6% (39.6 – 59.7%) sensitive thereafter. Among those who serially tested following an initial Ag-RDT-negative result, subsequent Ag-RDTs were 68.5% (62.0 – 74.3%) sensitive when performed at least 2 days later but only 34.3% (24.1 – 46.2%) sensitive before 2 days (p <0.001). Similarly, sensitivity was significantly higher with repeat testing at least 4 days after an initial Ag-RDT-negative result (75.8%, 68.4 – 81.9%), versus before 4 days (43.4%, 36.0 – 51.1%; p <0.001).

DISCUSSION

This prospective longitudinal study assessed characteristics associated with Ag-RDT performance in a highly vaccinated university population when the SARS-CoV-2 Omicron variant lineages predominated on-campus. Estimated Ag-RDT sensitivity and specificity were 53% and 99%, respectively, compared to rRT-PCR. Our findings suggest substantial differences in Ag-RDT performance by clinical characteristics and testing patterns. Sensitivity was notably higher for symptomatic (54%) versus asymptomatic (44%) testing and those with lower Ct values (i.e., higher SARS-CoV-2 loads; >80% sensitivity for Ct ≤20). Ag-RDT performance differed by testing order, where sensitivity was significantly higher for Ag-RDTs performed 1 day after rRT-PCR (69%), compared to 1 day before (38.3%).

Among symptomatic cases, sensitivity varied throughout the illness episode. We showed that Ag-RDTs do not sufficiently identify rRT-PCR-positive cases during early symptomatic illness

and that sensitivity peaked at 70% when the Ag-RDT was performed 4 to 7 days post-symptom onset. Sensitivity was <50% when Ag-RDTs were conducted before or the day of symptom onset. Negative concordance declined slightly in the days following rRT-PCR among symptomatic but not asymptomatic individuals, which may suggest those with discordant Ag-RDTs received an initial rRT-PCR-negative result in the days before SARS-CoV-2 became detectable and subsequently identified by Ag-RDTs. These findings highlight the importance of serial rapid antigen testing after an initial Ag-RDT-negative result, especially among recently symptomatic individuals or those with a high pre-test probability of infection (e.g., known SARS-CoV-2 exposure).[19–22]

The termination of the public health emergency in the United States on May 11, 2023 had repercussions on insurance coverage for COVID-19 testing, resulting in the elimination of cost-sharing.[23] This development underscores the significance of providing guidance regarding the most effective employment of Ag-RDT and identifying situations that may necessitate confirmatory rRT-PCR testing. CDC's current self-testing guidance recommends individuals test with an at-home antigen test immediately following onset of COVID-19 symptoms and at least 5 days after exposure to someone with COVID-19.[19,20,24] Serial testing is recommended for a total of two tests for symptomatic individuals and three tests for exposed individuals at 48-hour intervals following an initial negative result. Additionally, the self-testing guidance suggests benefits of testing in the absence of symptoms or known exposure to inform one's risk of transmitting SARS-CoV-2 to others.[20]

Our findings are consistent with the CDC self-testing guidance; however, low sensitivity observed for asymptomatic and recently symptomatic persons poses concerns about Ag-RDT effectiveness in controlling SARS-CoV-2 transmission chains. Current recommendations for serial testing may under-detect some recently symptomatic and asymptomatic SARS-CoV-2 infections. Given that the sensitivity of Ag-RDTs peaked at 4 to 7 days post-symptom onset in our study, extending the serial testing beyond 48 hours could be considered to increase detection of SARS-CoV-2 infections that initially test negative. In our study, serial testing sensitivity was significantly higher for Ag-RDTs repeated ≥2 days after an initial negative result (69%), compared to repeat testing before 2 days (34%). Additionally, there was only a 48% probability of detecting SARS-CoV-2 infection at least once out of all Ag-RDTs performed within 1 day among rRT-PCR-positive asymptomatic individuals, and the probability was comparable for all Ag-RDTs performed between days -1 and +7 (44%). Guidance regarding Ag-RDT use in the absence of symptoms or known SARS-CoV-2 exposure should be prefaced with information clarifying their reduced reliability in these groups.

Overall Ag-RDT sensitivity in our study did not meet the World Health Organization's minimum point-of-care performance criterion of 80% and was comparatively lower than prior studies conducted during Omicron emergence which reported overall sensitivities of approximately 80% and better performance in symptomatic individuals.[25,26] Potential reasons for lower Ag-RDT

performance in our study may include variation in test brands and lower viral loads among our highly-vaccinated study population. Higher estimated sensitivities of 70% and 75% reported in meta-analyses of studies conducted before 2022 may be partially due to the shedding dynamics of the Omicron lineages circulating during the study period and host factors such as COVID-19 vaccination and prior SARS-CoV-2 infection that contribute to reduced viral load in subsequent infection.[27–30] Reported patterns of pre-Omicron shedding dynamics suggest high transmissibility between 2 days before and 5 days after symptom onset, which initially led public health agencies to recommend a 10-day isolation period after the onset of symptoms.[31] However, recent studies which reported a 3-day incubation period for Omicron and serial intervals of 2- and 3-days for BA.1 and BA.2, respectively, compared to a 4-day serial interval and incubation period for Delta, show that emerging VOCs may exhibit distinct epidemiological characteristics.[10,32,33]

Similar to this analysis, several other studies have reported an association between lower Ct values and Ag-RDT concordance.[29,34] However, a low viral RNA copy number or high Ct may indicate a waning or escalating viral load trajectory, and this cannot be determined from a single measure. Diminished viral load may be observed during the early phase of SARS-CoV-2 infection prior to peak infectiousness and thus, detection of cases with higher Ct values and lower viral loads is an important component of preventing transmission to susceptible persons.[29] Several individual-level factors may contribute to variations in infectious virus shedding, such as heterogeneity in the neutralizing antibody response and viral genome load dynamics.[35] As more individuals acquire hybrid immunity from COVID-19 vaccination and one or more SARS-CoV-2 infections, more effective control of viral replication by the host immune system may explain the increased frequency of asymptomatic or mildly symptomatic infections.[29] However, the potential for asymptomatic or pauci-symptomatic transmission of SARS-CoV-2 to vulnerable populations in congregate settings, such as skilled nursing facilities, remains a public health risk. Thus, it may still be reasonable to recommend molecular rather than rapid antigen testing among certain groups for the protection of vulnerable persons. This may include continuing PCR-based testing for individuals in high-risk occupational settings, including among healthcare workers who may have been exposed to SARS-CoV-2 but exhibit minimal or no symptoms, and persons at risk of severe illness.

The study has limitations. This university-based study population was disproportionately vaccinated and aged 18 to 24 years, but under-representative of men and children compared to the general U.S. population. The sample of participants differed from the full study cohort in certain sociodemographic characteristics; most notably, fewer students (54% vs. 67% in the full cohort) and Greek sorority and fraternity members (4% vs 24% in the full cohort) were represented.[11] Participants who voluntarily self-reported test results were neither representative of the university overall nor the full cohort. While participants and individuals from the full cohort who did not report Ag-RDTs were socio-demographically comparable, they may differ by other characteristics related to Ag-RDT performance such as symptom severity (Supplemental

Table 5). Misclassification of unsupervised, self-reported symptoms, vaccine status, and Ag-RDT results may have occurred. However, prior studies have demonstrated Ag-RDT self-collection errors are not negatively associated with diagnostic accuracy and yield comparable sensitivities with professionally administered tests.[6,36] Although rRT-PCR samples were self-collected in our study, our group has previously demonstrated comparable quality of self-collected rRT-PCR samples to clinician-collected samples for detection of SARS-CoV-2.[37,38] Lastly, the generalizability of our findings may be limited to the Omicron SARS-CoV-2 lineages circulating at the time of investigation.

In conclusion, in this longitudinal study of over 5,000 individuals on a university campus, Ag-RDT performance with rRT-PCR varied by symptom status, time from symptom onset, and Orf1b Ct for detection of SARS-CoV-2 Omicron. Our findings support recommendations for repeat rapid antigen testing following an initial negative result among symptomatic individuals, until at least 4 days post-illness onset, and highlight the importance of re-evaluating rapid antigen diagnostic performance with the emergence of VOCs.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Table 1. Sociodemographic characteristics of study participants included in analysis from February – December 2022.

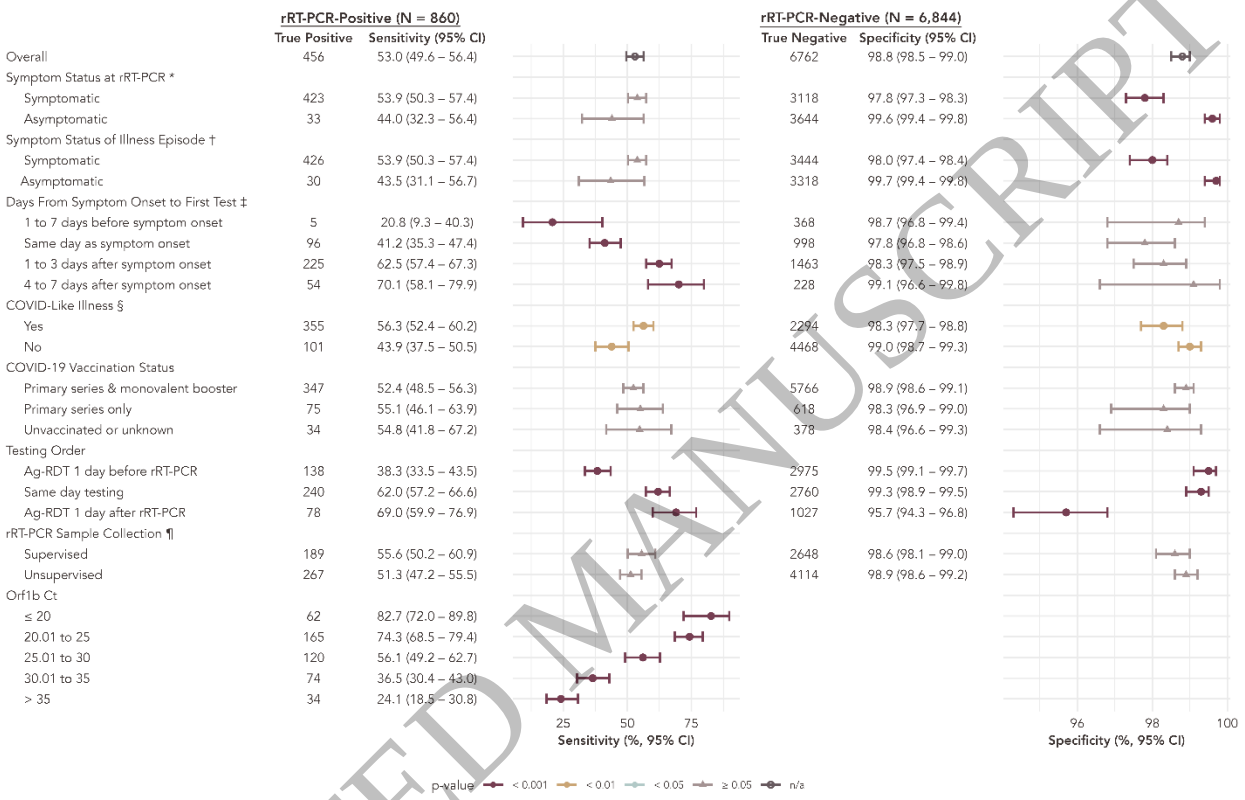
	No. (%) of participants, N = 5,575
Age, years (Median [Min, Max])	29.0 [18.0, 82.1]
Age, years (Mean [Std])	33.9 [15.0]
Age group	
18 – 24 years	2222 (39.9)
25 – 49 years	2279 (40.9)
≥ 50 years	1074 (19.3)
Sex assigned at birth	
Male	1830 (32.8)
Female	3708 (66.5)
Other or prefer not to say	37 (0.7)
Race	
American Indian or Alaskan Native	16 (0.3)
Asian	1419 (25.5)
Black or African American	89 (1.6)
Native Hawaiian or Pacific Islander	12 (0.2)
White	3359 (60.3)
Other	142 (2.6)
Multiple	418 (7.5)
Missing or prefer not to say	120 (2.2)

Hispanic/Latinx ethnicity	358 (6.4)
University affiliation	
Student	3026 (54.3)
Greek sorority or fraternity member	108 (3.6)
Staff	1746 (31.3)
Faculty	752 (13.5)
Other	51 (0.9)
Housing	
Off-campus house or apartment	2934 (52.6)
On-campus housing	848 (15.2)
Sorority or fraternity housing	11 (0.2)
Other	1782 (32.0)
Household size	
Lives alone	598 (10.7)
2 people	1486 (26.7)
3 – 5 people	1350 (24.2)
≥ 6 people	211 (3.8)
Missing	1930 (34.6)
On-campus frequency	
Do not come to campus	796 (14.3)
≤ 1 day/week	982 (17.6)
≥ 2 days/week	3796 (68.1)

Missing	1 (0.02)
Mask use	
Always	1547 (27.8)
Sometimes	1091 (19.5)
Never	44 (0.8)
Mixed*	2619 (47.0)
Missing	274 (4.9)
Social distancing	
Always	835 (15.0)
Sometimes	2136 (38.3)
Never	248 (4.5)
Mixed*	1976 (35.4)
Missing	380 (6.8)
COVID-19 vaccination status	
Fully vaccinated and boosted	4332 (77.7)
Fully vaccinated	800 (14.4)
Unvaccinated	39 (0.7)
Unknown	404 (7.3)

** Multiple responses of always, sometimes, and never reported during the study period.*

Figure 1. Sensitivity and specificity of SARS-CoV-2 antigen-detection rapid diagnostic tests (Ag-RDTs) performed within 1 day of real-time reverse transcription polymerase chain reaction (rRT-PCR) testing, N = 7,704 testing instances from 3,918 individuals, adjusted for intraparticipant correlation.



* Symptomatic if reported symptoms at least once with onset in the 7 days prior to or on rRT-PCR test date.

† Symptomatic if reported symptoms at least once with onset between the 7 days prior to and 7 days following rRT-PCR test date.

‡ First test date of each rRT-PCR-to-Ag-RDT match.

§ COVID-like illness indicated if participant reported acute onset of (1) at least one symptom of cough, loss of taste or smell, difficulty breathing or chest pain or (2) at least two symptoms of fever, chills, muscle or body aches, headache, sore throat, nausea or vomiting, diarrhea, fatigue, or runny nose within +/- 3 days of rRT-PCR.

¶ Supervised if in-person sample collection at a study testing site, and unsupervised if the sample was returned via dropbox or rapid mail courier.

Figure 2. Estimated positive predictive value (PPV) and negative predictive value (NPV) probabilities by SARS-CoV-2 prevalence at the overall antigen-detection rapid diagnostic test (Ag-RDT) sensitivity of 53.0% and specificity of 98.8%.

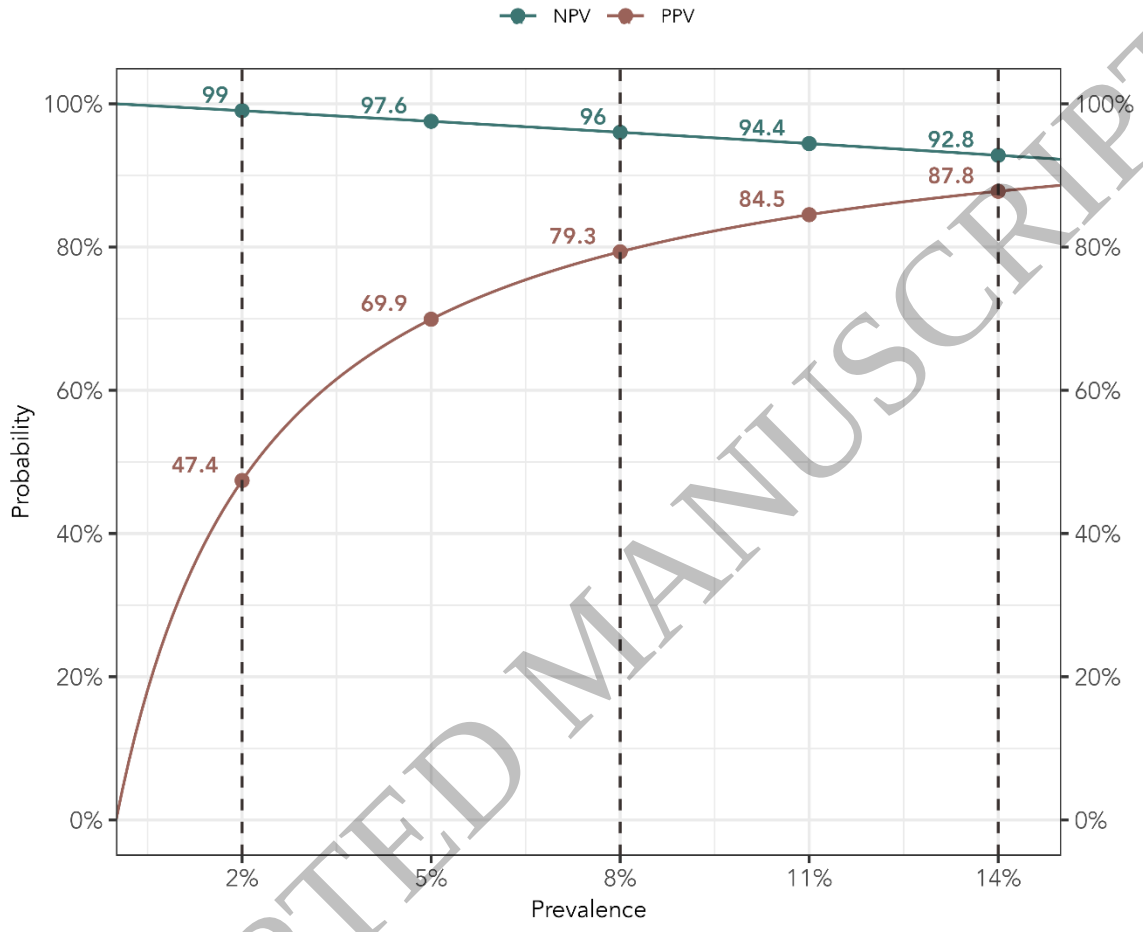


Figure 3. Distribution of Orf1b cycle threshold (Ct) as a surrogate marker for inverse of viral load by days from symptom onset to real-time reverse transcription polymerase chain reaction (rRT-PCR) among rRT-PCR-positives with an antigen-detection rapid diagnostic test (Ag-RDT) performed within 1 day, stratified by Ag-RDT result.

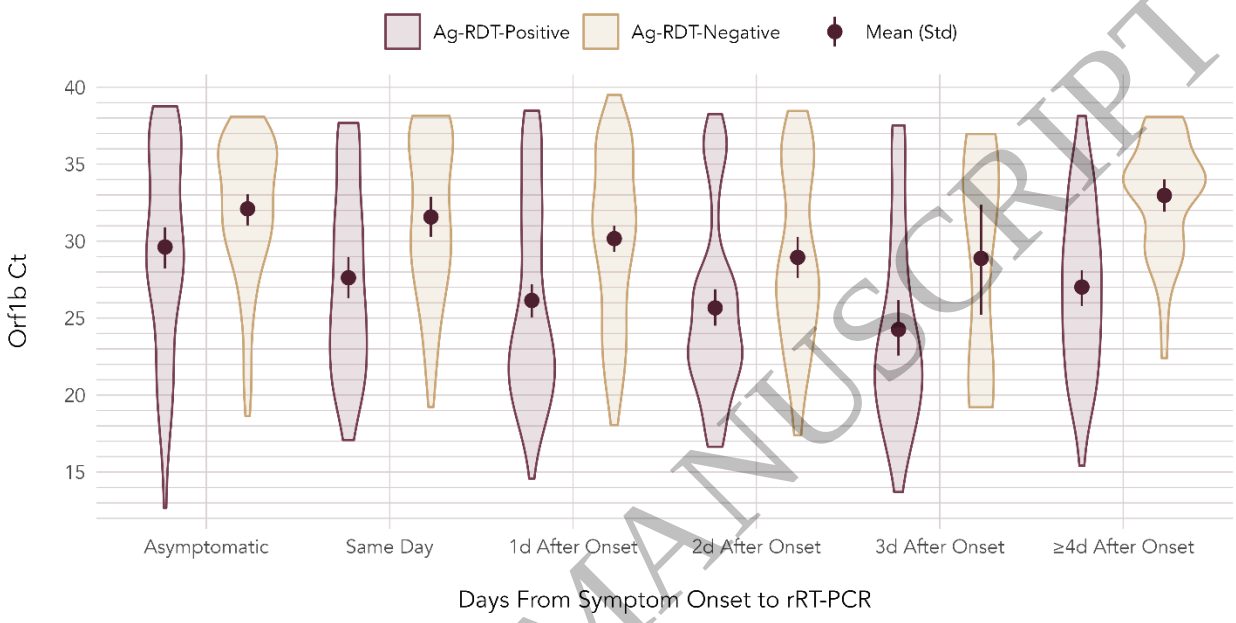
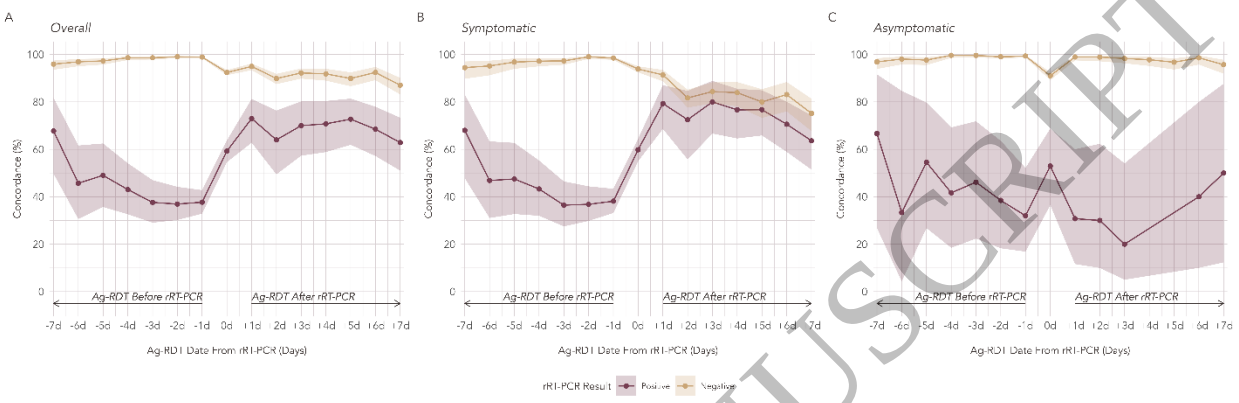


Figure 4a-c. SARS-CoV-2 antigen-detection rapid diagnostic test (Ag-RDT) concordance with real-time reverse transcription polymerase chain reaction (rRT-PCR) by difference in days between tests stratified by rRT-PCR test result and symptom status+, N = 17,572 rRT-PCR-to-Ag-RDT comparisons* from 5,575 individuals.



** Each Ag-RDT was matched to the closest rRT-PCR by test date within 7 days. 95% confidence intervals were adjusted for potential intraparticipant correlation using GEE methods.*

† Symptomatic includes individuals who reported any symptoms within 7 days of rRT-PCR testing.

Figure 5. SARS-CoV-2 antigen-detection rapid diagnostic test (Ag-RDT) serial testing among participants with at least one discordant Ag-RDT within 1 day of real-time reverse transcription polymerase chain reaction (rRT-PCR) positive test, N = 177 rRT-PCR-positive tests matched to 756 Ag-RDT results.

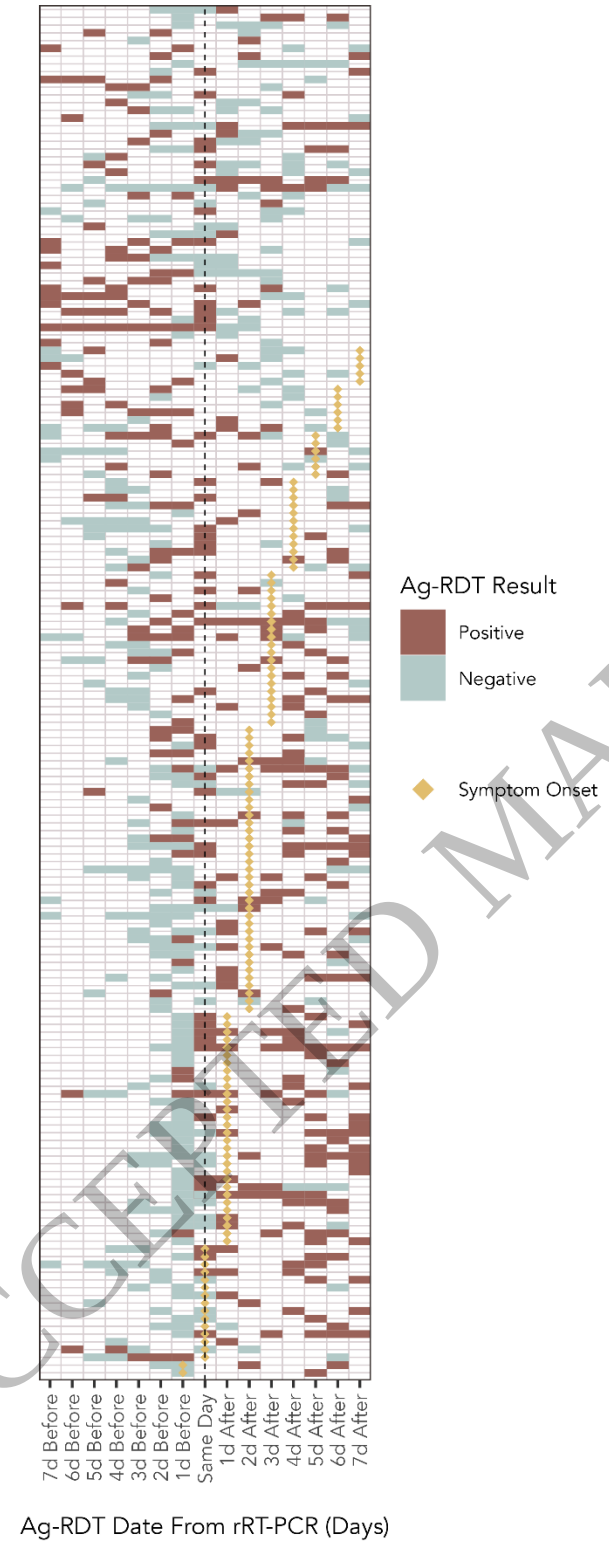
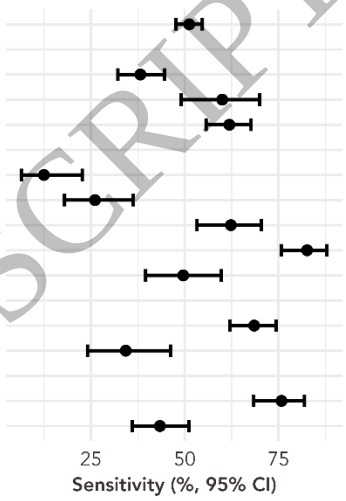


Figure 6. Sensitivity of serially-performed SARS-CoV-2 antigen-detection rapid diagnostic tests (Ag-RDTs) compared to real-time reverse transcription polymerase chain reaction (rRT-PCR) testing, N = 177 rRT-PCR-positive tests matched to 756 Ag-RDT results, adjusted for intraparticipant correlation.

	rRT-PCR-Positive (N = 177)	
	Total Ag-RDTs	Sensitivity (95% CI)
Overall	756	51.2 (47.7 – 54.7)
Testing Order *		
Ag-RDT before rRT-PCR	335	38.2 (32.2 – 44.6)
Same day testing	80	60.0 (49.1 – 70.0)
Ag-RDT after rRT-PCR	341	61.9 (55.7 – 67.7)
Ag-RDT Date From Symptom Onset *		
Before symptom onset	88	12.5 (6.5 – 22.7)
Same day as symptom onset	88	26.1 (18.0 – 36.3)
1 to 3 days after symptom onset	151	62.3 (53.3 – 70.4)
4 to 7 days after symptom onset	161	82.6 (75.7 – 87.9)
≥ 8 days after symptom onset	131	49.6 (39.6 – 59.7)
Serial Testing ≥ 2 Days After Initial Ag-RDT-Negative *		
Yes	355	68.5 (62.0 – 74.3)
No	67	34.3 (24.1 – 46.2)
Serial Testing ≥ 4 Days After Initial Ag-RDT-Negative *		
Yes	256	75.8 (68.4 – 81.9)
No	166	43.4 (36.0 – 51.1)



*p-value < 0.001

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