Screening for SARS-CoV-2 in close contacts of individuals with confirmed infection: performance and operational considerations

Stephanie Zobrist[1*#], Michelle Oliveira-Silva[2#], Alexia Martines Vieira[2], Pooja Bansil[1], Emily Gerth-Guyette[1], Brandon T Leader[1], Allison Golden[1], Hannah Slater[1], Catherine Duran de Lucena Cruz[2], Eduardo Garbin[2], Mariana Sagalovsky[1], Sampa Pal[1], Vin Gupta[3], Leo Wolansky[4], Deusilene Souza Vieira Dall'Acqua[5], Felipe Gomes Naveca[6], Valdinete Alves do Nascimento[6], Juan Miguel Villalobos Salcedo[5], Paul K Drain[7], Alexandre Dias Tavares Costa[8], Gonzalo J Domingo[1], Dhélio Pereira[2]

1. Diagnostics, PATH, Seattle, Washington, United States
2. Centro de Pesquisa em Medicina Tropical (CEPEM), Porto Velho, Rondônia, Brazil
3. Amazon.com, Seattle, Washington, United States
4. The Rockefeller Foundation, Pandemic Prevention Institute, New York City, New York, United States
5. Fundação Oswaldo Cruz (FIOCRUZ), Porto Velho, Rondônia, Brazil
6. Instituto Leônidas e Maria Deane (ILMD), Fundação Oswaldo Cruz (FIOCRUZ), Manaus, Amazonas, Brazil
7. Departments of Global Health and Medicine, University of Washington, Seattle, Washington, United States
8. Instituto Carlos Chagas (ICC), Fundação Oswaldo Cruz (FIOCRUZ), Curitiba, Paraná, Brazil

*Corresponding author. Stephanie Zobrist, Tel: 206-285-3500, Email: szobrist@path.org

[#] These authors contributed equally to this work.

Running head: SARS-CoV-2 screening considerations

Footnotes

Conflict of Interest Statement

The authors declare no conflicts of interest.

Funding Statement

This study was funded by The Rockefeller Foundation and Amazon.com.

Corresponding Author Contact Information

Stephanie Zobrist

2201 Westlake Avenue, Suite 200

Seattle, WA, USA 98121

Tel: 206-285-3500

Email: szobrist@path.org

Abstract

Background. Point-of-care and decentralized testing for SARS-CoV-2 is critical to inform public health responses. Performance evaluations in priority use cases such as contact tracing can highlight trade-offs in test selection and testing strategies.

Methods. A prospective diagnostic accuracy study was conducted among close contacts of COVID-19 cases in Brazil. Two anterior nares swabs (ANS), a nasopharyngeal swab (NPS), and saliva were collected at all visits. Vaccination history and symptoms were assessed. Household contacts were followed longitudinally. Three rapid antigen tests and one molecular method were evaluated for usability and performance against reference RT-PCR on NPS.

Results. Fifty index cases and 214 contacts (64 household) were enrolled. Sixty-five contacts were RT-PCR positive during at least one visit. Vaccination did not influence viral load. Gamma variants were most prevalent; Delta emerged increasingly during implementation. Overall sensitivity of evaluated tests ranged from 33%–76%. Performance was higher among symptomatic cases and cases with Ct<34 and lower among oligo/asymptomatic cases. Assuming a 24-hour time-to-result for RT-PCR, the cumulative sensitivity of an ANS rapid antigen test was >70% and almost 90% after four days.

Conclusions. The near immediate time-to-result for antigen tests significantly offsets lower analytical sensitivity in settings where RT-PCR results are delayed or unavailable.

Keywords: Porto Velho, Rondônia, Allplex™ SARS-CoV-2 Assay, SalivaDirect, SD Biosensor STANDARD Q COVID-19 Ag, LumiraDx SARS-CoV-2 Ag Test

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, which causes COVID-19, has significantly burdened health systems globally, with over 22 million confirmed cases in Brazil alone as of 2021 [1]. A key challenge of the pandemic response is access to appropriate diagnostic testing, which is critical to inform timely and targeted clinical management and public health strategies [2].

The reference standard for SARS-CoV-2 testing is RT-PCR. While accurate, this method has many practical limitations, including cost, laboratory infrastructure requirements, and often invasive sampling. RT-PCR testing is typically centralized, which can lead to delays in reporting results to patients. Such delays have important public health implications, including increased risk for transmission during the period before results are available to infected individuals [3,4]. Expanded access to decentralized and point-of-care (POC) testing is essential to identify cases early and limit community transmission, particularly where RT-PCR is unavailable.

Infected persons both with and without symptoms can transmit SARS-CoV-2 [5–7]. Due to the significance of asymptomatic transmission [8], testing these populations is often recommended, including close contacts of individuals with confirmed infection as part of contact tracing, testing, and isolation strategies [9,10]. However, contact tracing can be time and resource intensive, particularly during periods of high transmission, which can limit its implementation in practice.

Multiple platforms have been developed to enable decentralized and POC SARS-CoV-2 testing [11]. In particular, rapid antigen tests have garnered interest due to their lower cost, ease of use, and rapid turnaround time for results (typically under 30 minutes) [10, 12]. The World Health Organization (WHO) advises that rapid antigen tests meeting minimum performance criteria can be employed in a range of use cases, including for testing of asymptomatic contacts of cases [10]. Previous studies of rapid antigen test performance have shown variability, with strongest performance among symptomatic individuals with high viral loads in early stages of infection [11, 13–15]. Several studies have investigated test

performance among contacts of confirmed cases [16,17]; however, more data are needed to understand trade-offs in test selection and inform screening strategies regarding the timing and frequency of testing and performance characteristics.

Methods

Study design and population

A prospective diagnostic accuracy study was conducted among close contacts of COVID-19-positive index cases in Porto Velho, Brazil, between July and September 2021. Symptomatic adults within seven days of symptom onset who tested positive on a rapid SARS-CoV-2 antigen test (STANDARD Q COVID-19 Ag Nasal Test, SD Biosensor, Republic of Korea) were recruited as index cases through clinical platforms. Close contacts were identified through interviews administered at enrollment of the index case. Individuals 12 years of age or older who resided in Porto Velho were eligible for inclusion as close contacts if they met one or more of Brazil's criteria within the investigation period of the index case (two days prior to symptom onset to the time of the interview) (Supplementary Material A) [18]. Contacts with prior positive COVID-19 test results within the past three months were not eligible. A subset of household contacts (who shared a primary residence with the index case) had serial visits for clinical evaluations and testing every other day over nine days, for a total of up to five visits.

Tests evaluated

This study evaluated four SARS-CoV-2 tests: the STANDARD Q COVID-19 Ag Nasal and Saliva tests, the SARS-CoV-2 Ag Test (LumiraDx™ Limited, United Kingdom), and the SalivaDirect™ protocol (Yale School of Public Health, United States). The STANDARD Q tests are rapid chromatographic immunoassays for qualitative detection of antigens from SARS-CoV-2 in human nasal and saliva specimens, respectively. The LumiraDx test is a microfluidic immunofluorescence assay for qualitative detection of antigen in nasal specimens [19–24]. SalivaDirect is a dual-plexed RT-PCR method for SARS-CoV-2 detection from minimally processed saliva [25,26].

Study procedures at the point of care

At enrollment, information on participant demographics, health status, and medical history were collected. Presence, duration, and severity of symptoms were assessed at all visits. At each visit, two paired anterior nares swabs (ANS), one nasopharyngeal swab (NPS), and saliva were collected (Supplementary Material B). One ANS was used to run the STANDARD Q COVID-19 Ag Nasal Test during the visit. All specimens were then transferred to a laboratory where the remaining tests were performed.

For the longitudinal study, household contacts were followed every other day for up to five visits total, or until the POC screening test was positive. One additional visit was performed after this positive result, , during which NPS were not collected to minimize staff exposure. Participants were considered lost to follow-up after two missed visits.

Laboratory procedures

The extracted ANS mixed with LumiraDx buffer was frozen within five hours of collection and thawed before testing, no more than five days after freezing. The saliva sample was also frozen, and aliquots were thawed for testing with the STANDARD Q COVID-19 Ag Saliva Test (within five days of freezing) and the SalivaDirect assay. Evaluated tests were conducted per manufacturer instructions and by operators blinded to POC and reference results for close contacts.

Reference testing. NPS were used for reference testing with the Allplex™ SARS-CoV-2 Assay (Seegene Inc., Republic of Korea), a multiplex real-time PCR assay, on a CFX96 real-time PCR machine (Bio-Rad, United States) [27]. Automated RNA extraction was conducted using the Loccus Extracta kit (Loccus, Brazil). All SARS-CoV-2-positive specimens were repeated on the same assay for quantitative estimation of viral load. Specimens with cycle threshold (Ct) values <30 underwent genomic sequencing (Supplementary Material C). Staff conducting reference testing were blinded to the close contact results for tests under evaluation.

Usability assessment

Study staff responsible for use of the antigen tests were invited to participate in a usability assessment. A System Usability Scale (SUS) was employed, and an Ease of Use (EoU) questionnaire was adapted [21,28] (Supplementary Material D). SUS scores above 68 were considered acceptable [29,30]. To analyze data from the EoU questionnaire, a matrix was used to rank aspects of the products' usability as "satisfactory," "average," or "unsatisfactory" (Supplementary Material E) [21].

Sample size and statistical analysis

The sample size targeted at least 50 contacts with a positive reference result, including at least 20 asymptomatic individuals, to meet US FDA Emergency Use Authorization requirements [31].

Participants with no symptoms at the time of sampling were classified as asymptomatic. Participants were considered symptomatic if they presented with cough, shortness of breath, difficulty breathing, or at least two of the following symptoms at the time of sampling: fever, chills, rigor, myalgia, headache, sore throat, new olfactory or taste disorder [32]. Participants who presented with one or more mild symptoms but did not fit the symptomatic case definition and reported no care seeking or changes to behavior were considered oligosymptomatic.

Sensitivity, specificity, and positive and negative predictive values were calculated using standard formulas and presented with 95% CIs. Samples for which both RT-PCR and evaluated test results were available were included in the analysis. Using the longitudinal dataset, trade-offs between performance and utility of the evaluated tests in terms of cumulative sensitivity at specified time points were assessed as a function of time-to-results. Here, we use the term 'cumulative sensitivity' to refer to the probability that a rapid test will identify a SARS-CoV-2 positive individual at any point during the nine-day serial-testing follow-up period. For all household contacts in the longitudinal sample with a positive reference result (Ct<34) at any timepoint, time to positivity from the date of enrollment was evaluated as the

proportion of participants with a positive result by visit on a rapid test, as compared to the reference RT-PCR.

Data were collected and managed using REDCap electronic data capture tools hosted at the Institute of Translational Health Sciences [33]. Statistical analyses were conducted using Stata 15.0 (StataCorp, College Station, Texas, USA) and R 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical considerations

WCG Institutional Review Board (1301165), the CEPEM ethics committee, and Brazil's National Research Ethics Commission approved this study (44351421.0.0000.0011). Written informed consent was obtained for all participants. Minors under 18 provided assent, and written informed consent was obtained from parents/legal guardians.

Results

Participant characteristics

Fifty symptomatic COVID-19-positive index cases and 214 of their associated close contacts were enrolled (Table 1). Sixty-four contacts shared a primary residence with an index case and were therefore included in the longitudinal sample. Contacts ranged from ages 13 to 79. The majority of participants across all groups were female. Sixty-five contacts (30%, 65/214) were SARS-CoV-2 positive by the reference assay during at least one visit (Figure 1). For household contacts, positivity rates and symptom status varied by visit. Twenty-seven household contacts (42%, 27/64) tested positive by the reference test at the enrollment visit, 11 at visit 2 (28%, 11/39), 7 at visit 3 (20%, 7/35), 2 at visit 4 (6%, 2/33), and 5 at visit 5 (18%, 5/28). No SARS-CoV-2 positive household contacts presented with symptoms during visits 4 or 5 (Figure 1). In total, 42 paired samples were collected at unique visits with oligo/asymptomatic positive contacts, from 32 participants.

Vaccination status

Most participants were either partially (45%, 118/264) or fully (27%, 70/264) vaccinated at enrollment (Table 1). No statistical difference was observed in viral loads between vaccinated and unvaccinated individuals (Figure 2; Supplementary Material F, G).

Sequencing

Sequences were available for 84 positive samples: 68 Gamma (P.1, P.1.4, and P.1.7), and 16 Delta (AY.36, AY.4, AY.43, AY.99.2), with seven total lineages. The Delta strain became more prevalent among samples collected later in the study (Supplementary Material H).

Diagnostic performance

The two POC ANS antigen tests demonstrated comparable performance, with overall sensitivity of 55.0% for the STANDARD Q (95% CI 43.5%–66.2%) and 50.6% for LumiraDx (95% CI 39.1%–62.1%) (Table 2). Performance increased to >80% sensitivity for both tests among symptomatic cases but decreased to <30% among oligo/asymptomatic cases. For specimens with Ct values less than 34, above which viral viability is negligent and quantification is not as reliable [34,35], performance of both tests improved, with sensitivities in the ranges of 90% and 60% for symptomatic and oligo/asymptomatic cases, respectively.

The SalivaDirect PCR assay showed the highest overall performance at 75.9% sensitivity (95% CI 65.0%–84.9%), which increased to 88.2% (95% CI 76.1%–95.6%) among contacts with Ct<34. In all scenarios, the rapid STANDARD Q Saliva Test had a sensitivity of <60%, although performance increased among symptomatic positive cases at lower Ct levels.

Figure 3 presents the viral load of positive specimens, stratified by results of the STANDARD Q Nasal and Saliva tests. Overall, specimens with low viral loads were more likely to yield negative results; however, misclassification of specimens with high viral loads was more common with the saliva test.

Longitudinal analysis

To investigate how test results changed over time, descriptive grid plots were generated for all household contacts with a positive reference result at any timepoint (Supplementary Material I). Figure 4 includes two examples of overall patterns observed in the dataset: a) a symptomatic individual with a low Ct value who tested positive by all assays at the first visit and met the stopping criteria upon the second visit, and b) an individual with no or mild symptoms, and whose reference positivity status fluctuated between visits, with high Ct values overall, and no positive results on any rapid tests.

The time-to-positivity from days since enrollment for close contacts with a positive reference result (Ct<34) at any timepoint was assessed by comparing the proportion of participants with positive results by reference RT-PCR and a POC ANS antigen test (STANDARD Q Nasal) under different scenarios for RT-PCR result turnaround time (Figure 5). Even with a relatively rapid RT-PCR result turnaround of 24 hours, >70% of contacts would have been identified by a POC test. At 48 hours, cumulative sensitivity is 80%, increasing to nearly 90% at four days.

Usability

In total, 12 study staff completed the usability assessment. All three POC antigen tests were considered easy to use and SUS scores were acceptable (>77) (Supplementary Material J).

Discussion

In this study, performances of three POC antigen tests (two ANS and one saliva) and one molecular assay for SARS-CoV-2 in saliva were assessed among close contacts of COVID-19-positive index cases.

All evaluated tests demonstrated strongest performance among symptomatic cases—and particularly those with Ct values <34. Performance decreased among oligo/asymptomatic cases, which is consistent with results of prior studies [11,13] and may indicate that the tests are best able to detect those most likely

to be infectious [34,35]. However, there is no universal Ct value cut-off-point that corresponds to infectivity, and the relationship between Ct values and viral load varies by laboratory [11].

The SalivaDirect assay had the best performance, with sensitivity of up to 90% among contacts with Ct<34. Although this assay uses a noninvasive sample type and a simplified procedure that minimizes processing time and costs, infrastructure and training requirements still limit the feasibility of implementing this test in many settings, with potential implications for time-to-results.

The saliva antigen test had the lowest overall performance. Other evaluations of POC saliva antigen tests have also shown variable but generally sub-optimal performance [36,37]. One recent evaluation of this test reported an overall sensitivity of 66.1%; however, the reference assay was conducted on saliva [38]. In this study, the test was run on passively collected saliva. This may have impacted performance, as the manufacturer recommends use of actively collected saliva with snorted nasal mucus.

The two POC ANS antigen tests—STANDARD Q Nasal and LumiraDx—demonstrated comparable performance which was best among cases with Ct<34, with sensitivities in the ranges of 90% and 60% for symptomatic and asymptomatic cases, respectively. Among symptomatic cases and those with Ct<34, both tests met WHO performance criteria (≥ 80% sensitivity and ≥ 97% specificity) [10]. Both tests were also considered easy to use; however, the LumiraDx test requires the use of an instrument.

Overall, the observed positivity rate among close contacts in this study (65/214, 30%) highlights the importance of contact tracing and testing as a public health strategy [39]. The longitudinal data demonstrate the value of serial testing (particularly for individuals with known exposures) and the practical benefits of timely results [40, 41]. In this study, we show that in settings where RT-PCR is unavailable or where time-to-results is >4 days, close to 90% of individuals with Ct<34 could benefit from an earlier result via a POC test. Even in settings where RT-PCR results are available within 24 hours, cumulative sensitivity of a POC test is >70%. With repeat serial testing over a period of 9 days, the cumulative sensitivity of a POC ANS antigen test increases from 70% to near 90%. In many settings,

limited RT-PCR testing capacity—especially during high demand—can lead to delays in results. Immediate results can impact behavior of potentially infectious individuals, encouraging earlier isolation and signaling where additional testing is warranted [4]. The emergence of antiviral therapies—which are more effective the sooner they are taken—further underscores the value of timely results.

Limitations

Limitations of the study include its modest sample size, reflected in the 95% CIs reported with performance indicators. Further, the STANDARD Q Nasal and LumiraDx tests are among the best-in-class commercial POC antigen tests. Other tests with lower performance may increase the risk of missing infections against the benefit of identifying cases, to the extent that other strategies may be needed if RT-PCR is unavailable. Lastly, only Gamma and Delta variants were observed in this study; future research should investigate implications of new variants on diagnostic performance across sample types.

Conclusion

The near immediate time-to-result of rapid antigen tests is a significant benefit that offsets reduced sensitivity by decreasing diagnostic delays and onward viral transmission. Here, we demonstrate that POC ANS antigen tests for SARS-CoV-2 are easy to use and perform adequately to provide prompt, actionable information to both the health system and individuals.

Funding

This work was supported by grants from The Rockefeller Foundation [2020 HTH 039] and Amazon.com [2D-04020007] to GJD. REDCap hosted at the Institute of Translational Health Sciences is supported by the National Center For Advancing Translational Sciences of the National Institutes of Health under Award Number UL1 TR002319. FGN and VAN were supported by the National Council for Scientific and Technological Development [grant 403276/2020-9] and Inova Fiocruz/Fundação Oswaldo Cruz [grant VPPCB-007-FIO-18-2-30 - Knowledge generation]. FGN is a CNPq fellow.

Acknowledgements

The authors would like to thank all study participants as well as the clinical and laboratory staff at CEPEM involved with this study. We also thank SD Biosensor and LumiraDx for facilitating the availability of their tests for this study. Finally, we also thank Amanda Tsang and Christine Waresak for editorial support with the manuscript.

Tables

Table 1. Characteristics of study participants.

Characteristic	Index cases *(N=50)*	Close contacts, non-household *(N=150)*	Close contacts, household *(N=64)*
Age			
Mean (SD)	40.1 (12.8)	38.4 (14.6)	34.7 (17.2)
Range	19–68	13–86	14–79
Sex*, n (%)			
Female	32 (64.0)	81 (54.0)	37 (57.8)
Male	18 (36.0)	69 (46.0)	27 (42.2)
Vaccination status, n (%)**			
Fully vaccinated	12 (24.0)	43 (28.7)	15 (23.4)
Partially vaccinated	24 (48.0)	69 (46.0)	25 (39.0)
Unvaccinated	14 (28.0)	38 (25.3)	24 (37.5)
Vaccine type, n (%)			
AstraZeneca	19 (52.8)	47 (42.0)	11 (27.5)
CoronaVac	9 (25.0)	28 (25.0)	10 (25.0)
Johnson & Johnson	2 (5.6)	4 (3.6)	0 (0)
Pfizer	6 (16.7)	33 (29.5)	19 (47.5)
Relationship to index case, n (%)			
Family (same household)	-	0 (0)	64 (100.0)
Family (other household)	-	45 (29.3)	-
Neighbor	-	7 (4.7)	-
Friend	-	49 (32.7)	-

Coworker	-	41 (27.3)	-
Classmate	-	4 (2.7)	-
Other	-	4 (2.7)	-
Duration of estimated exposure, n (%)			
15 minutes to 1 hour	-	30 (20.0)	-
1 to 3 hours	-	41 (27.3)	-
3 to 8 hours	-	68 (45.3)	-
8+ hours	-	11 (7.4)	64 (100.0)
Location of exposure, n (%)			
Home	-	84 (56.0)	64 (100.0)
Work	-	47 (31.3)	-
Social setting	-	15 (10.0)	-
Other	-	4 (2.7)	-

* No statistical differences were observed by sex in any of the three groups, using a t-test.

** Fully vaccinated classification indicates that a participant had received all required vaccine doses and was >14 days since receipt of the last vaccine dose at enrollment.

Table 2. Performance indicators for tests evaluated using nasopharyngeal RT-PCR as the reference standard. Performance is shown across all close contacts and those with PCR Ct values of less than 34, 30, and 25. Tests with black headers were run on anterior nares swabs, and tests with grey headers were run on saliva.

	All close contacts			Close contacts (Ct<34)		
	Overall	Symptomatic positive	Oligo/asymptomatic positive	Overall	Symptomatic positive	Oligo/asymptomatic positive
STANDARD Q Nasal, n	**340**	**38**	**42**	**311**	**34**	**17**
Sensitivity (95% CI)	55.0 (43.5–66.2)	84.2 (68.8–94.0)	28.6 (15.7–44.6)	82.4 (69.1–91.6)	91.2 (76.3–98.1)	64.7 (38.3–85.8)
Specificity (95% CI)	100.0 (98.6–100.0)	N/A	N/A	100.0 (98.6–100.0)	N/A	N/A
PPV (95% CI)	100.0 (92.0–100.0)	N/A	N/A	100.0 (91.6–100.0)	N/A	N/A
NPV (95% CI)	87.8 (83.6–91.3)	N/A	N/A	96.7 (93.7–98.5)	N/A	N/A
LumiraDx Nasal, n	**345**	**37**	**42**	**316**	**33**	**17**
Sensitivity (95% CI)	50.6 (39.1–62.1)	81.1 (64.8–92.0)	23.8 (12.1–39.5)	78.0 (64.0–88.5)	87.9 (71.8–96.6)	58.8 (32.9–81.6)
Specificity (95% CI)	100.0 (98.6–100.0)	N/A	N/A	100.0 (98.6–100.0)	N/A	N/A
PPV (95% CI)	100.0 (91.2–100.0)	N/A	N/A	100.0 (91.0–100.0)	N/A	N/A
NPV (95% CI)	87.2 (82.9–90.7)	N/A	N/A	96.0 (93.0–98.0)	N/A	N/A
STANDARD Q Saliva, n	**340**	**38**	**42**	**311**	**34**	**17**
Sensitivity (95% CI)	32.5 (22.4–43.9)	50.0 (33.4–66.6)	16.7 (7.0–31.4)	47.1 (32.9–61.5)	52.9 (35.1–70.2)	35.3 (14.2–61.7)

Specificity (95% CI)	98.8 (96.7–99.8)	N/A	N/A	98.8 (96.7–99.8)	N/A	N/A
PPV (95% CI)	89.7 (72.6–97.8)	N/A	N/A	88.9 (70.8–97.6)	N/A	N/A
NPV (95% CI)	82.6 (78.0–86.7)	N/A	N/A	90.5 (86.5–93.6)	N/A	N/A
SalivaDirect RT-PCR, n	**339**	**38**	**41**	**311**	**34**	**17**
Sensitivity (95% CI)	75.9 (65.0–84.9)	89.5 (75.2–97.1)	63.4 (46.9–77.9)	88.2 (76.1–95.6)	94.1 (80.3–99.3)	76.5 (50.1–93.2)
Specificity (95% CI)	97.7 (95.0–99.1)	N/A	N/A	97.7 (95.0–99.1)	N/A	N/A
PPV (95% CI)	90.9 (81.3–96.6)	N/A	N/A	88.2 (76.1–95.6)	N/A	N/A
NPV (95% CI)	93.0 (89.3–95.8)	N/A	N/A	97.7 (95.0–99.1)	N/A	N/A
	Close contacts (Ct<30)			**Close contacts (Ct<25)**		
	Overall	**Symptomatic positive**	**Oligo/asymptomatic positive**	**Overall**	**Symptomatic positive**	**Oligo/asymptomatic positive**
STANDARD Q Nasal, n	**305**	**30**	**15**	**292**	**22**	**10**
Sensitivity (95% CI)	84.4 (70.5–93.5)	93.3 (77.9–99.2)	66.7 (38.4–88.2)	87.5 (71.0–96.5)	95.5 (77.2–99.9)	70.0 (34.8–93.3)
Specificity (95% CI)	100.0 (98.6–100.0)	N/A	N/A	100.0 (98.6–100.0)	N/A	N/A
PPV (95% CI)	100.0 (90.7–100.0)	N/A	N/A	100.0 (87.7–100.0)	N/A	N/A
NPV (95% CI)	97.4 (94.7–98.9)	N/A	N/A	98.5 (96.2–99.6)	N/A	N/A
LumiraDx	**310**	**29**	**15**	**297**	**21**	**10**

Nasal, n						
Sensitivity (95% CI)	79.5 (64.7–90.2)	89.7 (72.7–97.8)	60.0 (32.3–83.7)	87.1 (70.2–96.4)	100.0 (83.9–100.0)	60.0 (26.2–87.8)
Specificity (95% CI)	100.0 (98.6–100.0)	N/A	N/A	100.0 (98.6–100.0)	N/A	N/A
PPV (95% CI)	100.0 (90.0–100.0)	N/A	N/A	100.0 (87.2–100.0)	N/A	N/A
NPV (95% CI)	96.7 (93.9–98.5)	N/A	N/A	98.5 (96.3–99.6)	N/A	N/A
STANDARD Q Saliva, n	**305**	**30**	**15**	**292**	**22**	**10**
Sensitivity (95% CI)	48.9 (33.7–64.2)	53.3 (34.3–71.7)	40.0 (16.3–67.7)	50.0 (31.9–68.1)	59.1 (36.4–79.3)	30.0 (6.7–65.3)
Specificity (95% CI)	98.8 (96.7–99.8)	N/A	N/A	98.8 (96.7–99.8)	N/A	N/A
PPV (95% CI)	88.0 (68.8–97.5)	N/A	N/A	84.2 (60.4–96.6)	N/A	N/A
NPV (95% CI)	91.8 (87.9–94.7)	N/A	N/A	94.1 (90.7–96.6)	N/A	N/A
SalivaDirect RT-PCR, n	**305**	**30**	**15**	**292**	**22**	**10**
Sensitivity (95% CI)	88.9 (75.9–96.3)	96.7 (82.8 – 99.9)	73.3 (44.9 – 92.2)	87.5 (71.0–96.5)	95.5 (77.2–99.9)	70.0 (34.8–93.3)
Specificity (95% CI)	97.7 (95.0–99.1)	N/A	N/A	97.7 (95.0–99.1)	N/A	N/A
PPV (95% CI)	87.0 (73.7–95.1)	N/A	N/A	82.4 (65.5–93.2)	N/A	N/A
NPV (95% CI)	98.1 (95.6–99.4)	N/A	N/A	98.4 (96.1–99.6)	N/A	N/A

1 CI: confidence interval; Ct: cycle threshold; PPV: positive predictive value; NPV: negative predictive value; N/A: not applicable; RT-PCR:
2 reverse transcription-polymerase chain reaction

References

1. Ministry of Health Health Surveillance Secretariat. Special Epidemiological Bulletin: Coronavirus Disease COVID-19. 10 December 2021. Available at: https://www.gov.br/saude/pt-br/media/pdf/2021/dezembro/11/boletim_epidemiologico_covid_92_10dez21.pdf.
2. Vandenberg O, Martiny D, Rochas O, van Belkum A, Kozlakidis Z. Considerations for diagnostic COVID-19 tests. Nat Rev Microbiol **2021** ; 19(3):171-183. doi:10.1038/s41579-020-00461-z.
3. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert JHHM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. Lancet Public Health **2020** ; 5(8):e452-e459. doi:10.1016/S2468-2667(20)30157-2.
4. Larremore DB, Wilder B, Lester E, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. Sci Adv **2021** ; 7(1):eabd5393. doi:10.1126/sciadv.abd5393.
5. Sah P, Fitzpatrick MC, Zimmer CF, et al. Asymptomatic SARS-CoV-2 infection: a systematic review and meta-analysis. Proc Natl Acad Sci U S A **2021** ; 118(34):e2109229118. doi:10.1073/pnas.2109229118.
6. Buitrago-Garcia D, Egli-Gany D, Counotte MJ, et al. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: a living systematic review and meta-analysis. PLoS Med **2020** ; 17(9):e1003346. doi:10.1371/journal.pmed.1003346.
7. Byambasuren O, Cardona M, Bell K, et al. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis. JAMMI **2020** ; 5(4): 223–234. doi: 10.3138/jammi-2020-0030.
8. Qiu X, Nergiz AI, Maraolo AE, et al. The role of asymptomatic and pre-symptomatic infection in SARS-CoV-2 transmission- a living systematic review. Clin Microbiol Infect **2021** ; 27(4): 511–519. doi: 10.1016/j.cmi.2021.01.011.

9. Fiore VG, DeFelice N, Glicksberg BS, et al. Containment of COVID-19: Simulating the impact of different policies and testing capacities for contact tracing, testing, and isolation. PLoS One **2021** ; 16(3):e0247614. doi:10.1371/journal.pone.0247614.
10. World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection. Interim Guidance. 6 October 2021. Geneva; WHO. Available at: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays.
11. Dinnes J, Deeks JJ, Adriano A, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev **2020** ; 8(8):CD013705. doi:10.1002/14651858.CD013705.
12. Drain P. Rapid diagnostic testing for SARS-CoV-2 [published online ahead of print, 2022 Jan 7]. N Eng J Med **2022.** doi:10.1056/NEJMcp2117115.
13. Brümmer LE, Katzenschlager S, Gaeddert M, et al. Accuracy of novel antigen rapid diagnostics for SARS-CoV-2: a living systematic review and meta-analysis [published correction appears in PLoS Med **2021** Oct 13 ; 18(10):e1003825]. PLoS Med **2021** ; 18(8):e1003735. doi:10.1371/journal.pmed.1003735.
14. Boum Y, Fai KN, Nikolay B, et al. Performance and operational feasibility of antigen and antibody rapid diagnostic tests for COVID-19 in symptomatic and asymptomatic patients in Cameroon: a clinical, prospective, diagnostic accuracy study [published correction appears in Lancet Infect Dis **2021 Jun** ; 21(6):e148] [published correction appears in Lancet Infect Dis. **2021 Jul** ; 21(7):e182]. Lancet Infect Dis **2021** ; 21(8):1089-96. doi:10.1016/S1473-3099(21)00132-8.
15. Baro B, Rodo P, Ouchi D, et al. Performance characteristics of five antigen-detecting rapid diagnostic test (Ag-RDT) for SARS-CoV-2 asymptomatic infection: a head-to-head benchmark comparison. J Infect **2021** ; 82(6):269 -275. doi: 10.1016/j.jinf.2021.04.009

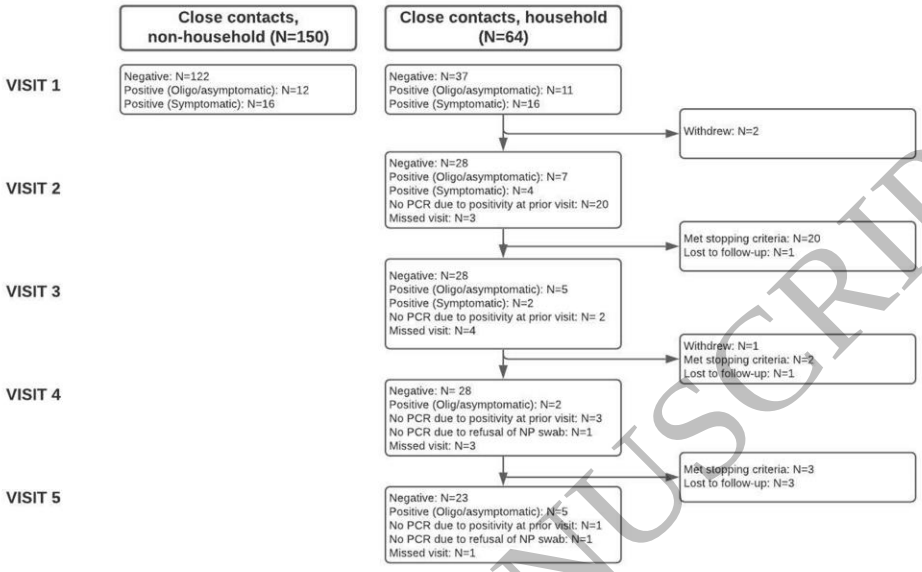
16. Torres I, Poujois S, Albert E, Colomina J, Navarro D. Evaluation of a rapid antigen test (Panbio™ COVID-19 Ag rapid test device) for SARS-CoV-2 detection in asymptomatic close contacts of COVID-19 patients. Clin Microbiol Infect **2021** ; 27(4):636.e1-636.e4. doi:10.1016/j.cmi.2020.12.022.
17. Schuit E, Veldhuijzen IK, Venekamp RP, et al. Diagnostic accuracy of rapid antigen tests in asymptomatic and presymptomatic close contacts of individuals with confirmed SARS-CoV-2 infection: cross sectional study. BMJ **2021** ; 374:n1676. doi:10.1136/bmj.n1676.
18. Ministry of Health. Epidemiological Surveillance Guide: Public Health Emergency of National Importance due to Coronavirus Disease 2019. Available at: https://portalarquivos.saude.gov.br/images/af_gvs_coronavirus_6ago20_ajustes-finais-2.pdf.
19. Drain P, Sulaiman R, Hoppers M, Lindner NM, Lawson V, Ellis JE. Performance of the LumiraDx Microfluidic Immunofluorescence Point-of-Care SARS-CoV-2 Antigen Test in asymptomatic adults and children [published online ahead of print, 2021 Oct 20]. Am J Clin Pathol **2021** ; aqab173. doi:10.1093/ajcp/aqab173.
20. Kohmer N, Toptan T, Pallas C, et al. The comparative clinical performance of four SARS-CoV-2 rapid antigen tests and their correlation to infectivity in vitro. J Clin Med **2021** ; 10(2):328. doi:10.3390/jcm10020328.
21. Krüger LJ, Klein JAF, Tobian F, et al. Evaluation of accuracy, exclusivity, limit-of-detection and ease-of-use of LumiraDx™: An antigen-detecting point-of-care device for SARS-CoV-2 [published online ahead of print, 2021 Aug 12]. Infection **2021** ; 1-12. doi:10.1007/s15010-021-01681-y.
22. Bianco G, Boattini M, Barbui AM, et al. Evaluation of an antigen-based test for hospital point-of-care diagnosis of SARS-CoV-2 infection. J Clin Virol **2021** ; 139:104838. doi:10.1016/j.jcv.2021.104838.

23. Fekete T. In adults and children, a rapid POC antigen test for COVID-19 (LumiraDx) had ≥97% sensitivity and specificity vs. RT-PCR. Ann Intern Med **2021** ; 174(7):JC82. doi:10.7326/ACPJ202107200-082.
24. Karon BS, Donato LJ, Bridgeman AR, et al. Analytical sensitivity and specificity of four point of care rapid antigen diagnostic tests for SARS-CoV-2 using real-time quantitative PCR, quantitative droplet digital PCR, and a mass spectrometric antigen assay as comparator methods. Clin Chem **2021** ; 67(11):1545-1553. doi:10.1093/clinchem/hvab138.
25. Vogels CBF, Watkins AE, Harden CA, et al. SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. Med (N Y) **2021** ; 2(3):263-280.e6. doi:10.1016/j.medj.2020.12.010.
26. Rodríguez Flores SN, Rodríguez-Martínez LM, Reyes-Berrones BL, Fernández-Santos NA, Sierra-Moncada EJ, Rodríguez-Pérez MA. Comparison between a standard and SalivaDirect RNA extraction protocol for molecular diagnosis of SARS-CoV-2 using nasopharyngeal swab and saliva clinical samples. Front Bioeng Biotechnol **2021** ; 9:638902. doi:10.3389/fbioe.2021.638902.
27. Freppel W, Merindol N, Rallu F, Bergevin M. Efficient SARS-CoV-2 detection in unextracted oro-nasopharyngeal specimens by rRT-PCR with the Seegene Allplex™ 2019-nCoV assay. Virol J **2020** ; 17(1):196. doi: 10.1186/s12985-020-01468-x.
28. World Health Organization. Antigen detection rapid diagnostic tests for coronavirus disease 2019 (COVID-19): master protocol for monitored implementation. Version 1.0. Geneva: WHO, **25 November 2020**. Available at: https://www.who.int/news-room/articles-detail/sars-cov-2-antigen-detecting-rapid-diagnostic-test-implementation-proposals.
29. Sauro, J. A practical guide to the System Usability Scale: Background, benchmarks, & best practices. Denver, CO: Measuring Usability LLC, **2011**.

30. Bangor, A., Kortum, PT, & Miller, JT. (2008). An empirical evaluation of the System Usability Scale. Int J Hum Comput Interact **2008** ; 24(6), 574–94. https://doi.org/10.1080/10447310802205776.
31. United States Food and Drug Administration. Template for Developers of Antigen Tests. Available at: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas. Version October 26 2020. Accessed 24 November 2020.
32. Centers for Disease Control and Prevention. Coronavirus Disease 2019 (COVID-19) 2020 Interim Case Definition. Available at: https://ndc.services.cdc.gov/case-definitions/coronavirus-disease-2019-2020/. Accessed 10 September 2021.
33. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support, J Biomed Inform **2009** ; Apr;42(2):377-81.
34. Routsias JG, Mavrouli M, Tsoplou P, Dioikitopoulou K, Tsakris A. Diagnostic performance of rapid antigen tests (RATs) for SARS-CoV-2 and their efficacy in monitoring the infectiousness of COVID-19 patients. Sci Rep **2021** ; 11:22863.
35. La Scola B, Le Bideau M, Andreani J, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis **2020** ; 39:1059–61.
36. Yokota I, Sakurazawa T, Sugita J, et al. Performance of qualitative and quantitative antigen tests for SARS-CoV-2 using saliva. Infect Dis Rep **2021** ; 13(3):742-7. doi:10.3390/idr13030069.
37. Kritikos A, Caruana G, Brouillet R, et al. Sensitivity of rapid antigen testing and RT-PCR performed on nasopharyngeal swabs versus saliva samples in COVID-19 hospitalized patients: results of a prospective comparative trial (RESTART). Microorganisms **2021** ; 9(9):1910. doi:10.3390/microorganisms9091910.

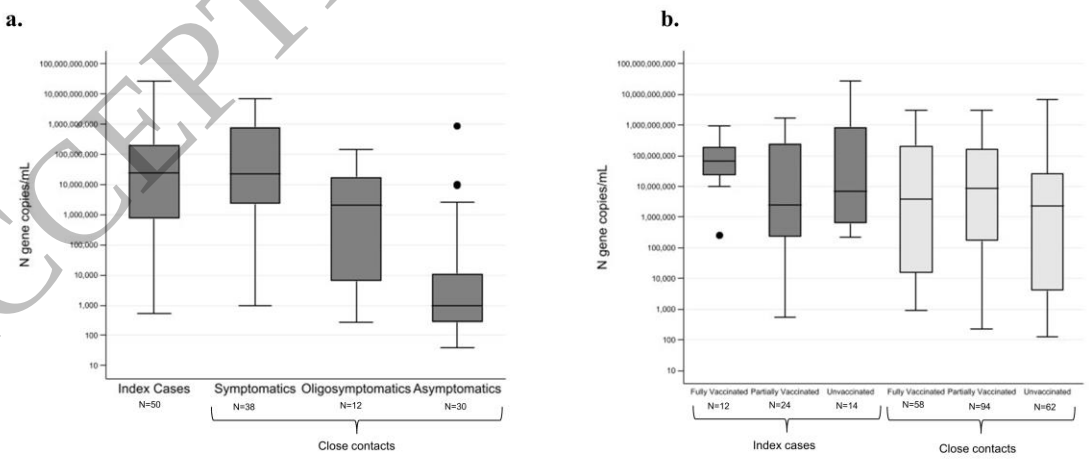
38. Igloi Z, Velzing J, Huisman R, et al. Clinical evaluation of the SD Biosensor saliva antigen rapid test with symptomatic and asymptomatic, non-hospitalized patients. PLoS One **2021** ; Dec 22;16(12):e0260894. doi:10.1371/journal.pone.0260894.
39. Chung S, Marlow S, Tobias N, et al. Lessons from countries implementing find, test, trace, isolation and support policies in the rapid response of the COVID-19 pandemic: a systematic review. BMJ Open **2021** ; 11(7):e047832. Doi: 10.1136.bmjopen-2020-047832.
40. Smith RL, Gibson LL, Martinez PP, et al. Longitudinal assessment of diagnostic test performance over the course of acute SARS-CoV-2 infection. J Infect Dis **2021** ; 224(6). doi:10.1093/infdis/jiab337.
41. Revollo B, Blanco I, Soler P, et al. Same-day SARS-CoV-2 antigen test screening in an indoor mass-gathering live music event: a randomized controlled trial. Lancet Infect Dis **2021** ; 21(10):P1365 – 1372. doi: 10.1016/S1473-3099(21)00268-1

Figure 1. Status of study participants, by visit.



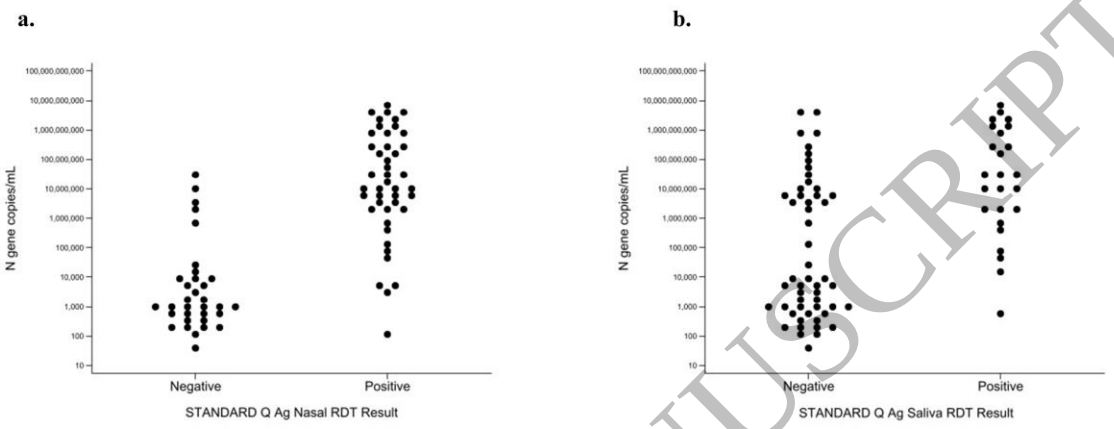
165x93 mm (1.1 x DPI)

Figure 2. Viral load value relationships of study participants a) by infection category and b) by vaccination status.



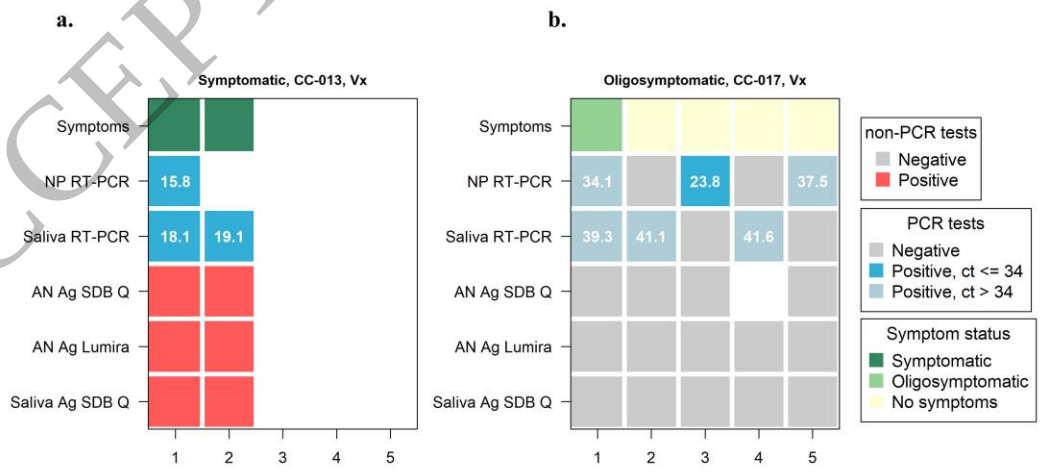
165x93 mm (1.1 x DPI)

Figure 3. Viral load value distributions across antigen tests among close contacts for a) the STANDARD Q Nasal Test and b) the STANDARD Q Saliva Test.



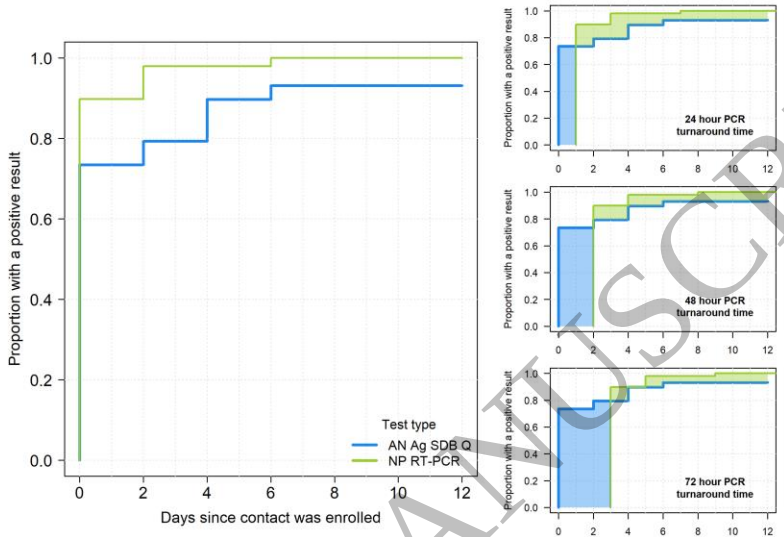
165x93 mm (1.1 x DPI)

Figure 4. Descriptive plots for a subset of close contacts positive by the RT-PCR reference assay. Visit numbers are shown on the x-axis, and test results and symptom status are shown on the y-axis. Symptom status is presented independently of RT-PCR reference assay result.



165x93 mm (1.1 x DPI)

Figure 5. Time to positivity from time of first visit for close contacts with a positive NPS RT-PCR result (Ct<34) at any time. The blue line represents the proportion of NPS RT-PCR positive cases identified as positive by the point-of-care antigen test (STANDARD Q COVID-19 Ag test) on nasal samples, and the green line represents those identified by the reference NPS RT-PCR. Four different scenarios for RT-PCR result turnaround times are presented.



165x93 mm (1.1 x DPI)