ELSEVIER

Virology

Detection of SARS-CoV-2 infection in asymptomatic populations using the DiaSorin molecular Simplexa and Roche Cobas EUA assays

Check for updates

Stella Antonara[a,b,*], Patrick Ozbolt[a], Lorie Landon[a], Lisa Fatica[a], Tamra Pleasant[a], Juanita Swickard[a], Andrew Drury[a], Nicholas Wongchaowart[a,b], Kendall W. Cradic[a,b]

[a] OhioHealth Laboratory Services, OhioHealth, Columbus, OH, USA
[b] CORPath Pathology Services, LLC, Columbus, OH, USA

A B S T R A C T

Identification of asymptomatic patients is necessary to control the COVID-19 pandemic and testing is one of the measures to detect this population. We evaluated the clinical correlation of the DiaSorin Molecular Simplexa COVID-19 Direct (DiaSorin Molecular) and Roche Cobas 6800 SARS-CoV-2 (Roche) assays using 253 oropharyngeal (OP) swab specimens collected from asymptomatic patients. Agreement between DiaSorin Molecular and Roche was 97% (95% CI, 0.94 to 0.99), with a κ statistic of 0.90 (95% CI, 0.83 to 0.97) and a PPA of 89% (95% CI, 0.76 to 0.96) and NPA of 99% (95% CI, 0.97 to 0.99). Simple regression analysis of Ct values revealed a regression line of y = 1.065*X - 5.537 with a Pearson's r of 0.8542, indicating a good correlation between both platforms. The DiaSorin Molecular assay demonstrates clinical performance comparable to that of Roche in this population.

© 2021 Elsevier Inc. All rights reserved.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases continue to grow worldwide since the first cases of coronavirus disease 2019 (COVID-19) were observed in December 2019 in Wuhan, China (Anon; WHO 2020). Much remains unknown about SARS-CoV-2; however, the presence of asymptomatic COVID-19 cases was noted in early reports of the outbreak (Bai et al., 2020; Chan et al., 2020; He et al., 2020; Rothe et al., 2020). For instance, a case study of a family in China showed how SARS-CoV-2 spread from an asymptomatic carrier to 5 other family members in other locations while she remained asymptomatic for the entire 21-day follow-up period (Bai et al., 2020). Similarly, the asymptomatic proportion was estimated to be 17.9% among those who tested positive for SARS-CoV-2 onboard the Diamond Princess cruise ship (Mizumoto et al., 2020). Asymptomatic transmission has been described previously for coronaviruses and other respiratory viruses such as Middle East respiratory syndrome coronavirus (MERS-CoV), human rhinoviruses (HRVs), influenza virus, and severe acute respiratory syndrome coronavirus (SARS-CoV) (Al-Tawfiq and Gautret, 2019; Furuya-Kanamori et al., 2016; Granados et al., 2015; Wilder-Smith et al.,

2005; Yuen et al., 2020). While the prevalence of asymptomatic individuals with SARS-CoV-2 is not yet fully characterized, it is estimated that approximately 45% of those infected with SARS-CoV-2 will remain asymptomatic (Oran and Topol, 2020). It has been challenging to accurately quantify the prevalence of asymptomatic individuals including limitations of previous study designs such as population size, longitudinal data, and distinguishing truly asymptomatic cases from those who are merely pre-symptomatic. Hence, the occurrence of asymptomatic patients with SARS-CoV-2 infections poses a significant threat to COVID-19 control efforts and may have been a critical factor in community spread (Al-Tawfiq, 2020; Lu et al., 2020; McArthur et al., 2020). In fact, recent studies have shown that in some cases, the detection pattern and viral load of infected asymptomatic individuals have been equal to that of symptomatic individuals, suggesting a similar likelihood of viral transmission (Kimball et al., 2020; Walsh et al., 2020; Zou et al., 2020).

Molecular diagnostic testing has been critical in identifying infected symptomatic cases during the current pandemic. However, as lockdown measures have started to ease, Emergency Use Authorization (EUA) *in vitro* diagnostic tests for SARS-CoV-2 are being used to assess symptomatic patients and asymptomatic individuals to facilitate a return to normalcy. Recent guidelines from the Centers for Disease Control and Prevention (CDC) recommend using available EUA *in vitro* diagnostic tests for SARS-Cov-2 to diagnose acute infection of both symptomatic and asymptomatic individuals, to guide contact tracing and treatment options, pre-operative

testing, and isolation requirements (CDC, 2021). From a healthcare setting perspective, it is imperative that testing programs include asymptomatic testing since identifying individuals with suspected COVID-19 is critical for real-time cohorting decisions and infection control measures.

Accurate and reliable results are necessary for correctly identifying asymptomatic patients since molecular assays with low clinical sensitivity and specificity for asymptomatic populations may inevitably lead to more exposures and have profound safety implications for the public health and healthcare systems. Simultaneously, to meet the exponential demand in testing, there has been accelerated development of both molecular and serological assays across a plethora of platforms (FDA 2021; La Marca et al., 2020). The selection from over 200 molecular diagnostic assays and counting requires understanding their limitations in diagnostic performance and further data-based clarification of their testing utility with different COVID-19 populations. Therefore, in this study, we evaluated the clinical performance of the DiaSorin Molecular Simplexa COVID-19 Direct and Roche Cobas 6800 SARS-CoV-2 EUA assays using oropharyngeal swabs from asymptomatic patients. This information may provide significant insights to support population screening strategies and the use of these two assays for the detection of SARS-CoV-2 in asymptomatic patients, providing the rapid detection needed for diagnosis, isolation, and contact tracing of COVID-19 cases.

2. Materials and methods

2.1. Specimen collection and storage

Flocked swabs were used to collect oropharyngeal specimens from asymptomatic patients with known exposures to suspected COVID-19 infections or for pre-operative testing for surgical patients at OhioHealth Riverside Methodist Hospital (Columbus, OH). After collection, the swabs were placed into 3 ml of sterile Universal Viral Transport (UVT; BD). Specimens were tested as soon as possible after collection, or if testing was delayed, were stored for up to 72 h at 2-8° C. Following routine testing, samples were stored frozen ($\leq$-80°C) until comparator testing could be completed.

2.2. Study design

Assay performance was evaluated using a total of 253 oropharyngeal specimens originally submitted for routine COVID-19 testing at OhioHealth Riverside Methodist Hospital on the Roche Cobas SARS-CoV-2 assay. Positive and negative specimens were selected during the submission process for EUA approval of the Roche assay for use in asymptomatic patients. Samples were thawed and subsequently tested on the DiaSorin Molecular Simplexa COVID-19 Direct assay. The study population included patients 4-85 years of age and both genders and were selected based on the patient's status (asymptomatic individual reported on the order entry questions).

2.3. Roche Cobas SARS-CoV-2

The Roche assay (Roche Diagnostics, Indianapolis, IN) was performed according to the manufacturer's instructions for use. The test uses a minimum required sample volume of 600 μL. The sample preparation is fully automated (nucleic acid extraction and purification) followed by RT-PCR amplification and detection. The assay targets the ORF1 a/b non-structural region that is unique to SARS-CoV-2. Additionally, Cobas targets a conserved region in the structural protein envelope E-gene with pan-Sarbecovirus (PAN-SARS) detection that will also detect the SARS-CoV-2 virus. The result was interpreted as positive if both targets were detected and presumptive positive if one of two targets was detected.

2.4. DiaSorin Molecular Simplexa COVID-19 Direct

The Simplexa COVID-19 Direct assay (DiaSorin Molecular, Cypress, CA) was performed according to the manufacturer's instructions for use. Briefly, 50 μL of Simplexa COVID-19 Direct Kit reaction mix (MOL4150) was added to the "R" well of the 8-well Direct Amplification Disc (DAD) followed by adding 50 μL of non-extracted oropharyngeal swab sample (collected in approximately 3 mL of UVT (UVT, BD)) to the "SAMPLE" well. Tests were run on the LIAISON MDX system, and data collection and analysis were performed with LIAISON MDX Studio software. The assay targets two different regions of the SARS-CoV-2 genome, the S gene, and ORF1ab, differentiated with FAM and JOE fluorescent probes. An RNA internal control (Q670 probe) is used to detect RT-PCR failure and or inhibition. The result interpretation algorithm for reporting a positive specimen requires only one of the two targets to be detected (S or ORF1ab gene). The oropharyngeal specimen is off-label for DiaSorin Molecular, but proper validation was conducted and published previously by the OhioHealth Laboratory Services (Cradic et al., 2020).

2.5. Discordant analysis

Results were considered discordant when the DiaSorin Molecular assay did not agree qualitatively (Detected or Not Detected) with Roche Cobas results. In such cases, molecular testing was repeated for the discordant assay when a remnant sample was available, and a retrospective chart review was conducted.

2.6. Statistical methods

Positive percent agreement (PPA) and negative percent agreement (NPA) between both assays were calculated with two-sided (upper/lower) 95% confidence intervals (CIs) using the Evidence-Based Medicine Toolbox, Knowledge Translation Program (Toronto, CA). The percent positive agreement was calculated as TP/(TP + FN) x 100 and the percent negative agreement was calculated as TN/(TN + FP) x 100, where TP were true-positive results, FN were false-negative results, TN were true-negative results, and FP were false-positive results. Cohen's kappa values (κ) were also calculated as a measure of overall agreement, with values categorized as almost-perfect (>0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal (0.21 to 0.39), or none (0 to 0.20) (Landis and Koch, 1977; McHugh, 2012). The discordance rate was calculated as (FP + FN)/Total Number of Samples tested X 100. Simple regression, Pearson's correlation coefficient (Pearsons r), Bland-Altman plot, and nested ANOVA analysis on the cycle threshold (Ct) values were performed using GraphPad Prism version 8.4.3 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

3. Results

3.1. Clinical performance of DiaSorin Molecular Simplexa COVID-19 Direct for asymptomatic patients

Following testing of 253 specimens, DiaSorin Molecular and Roche's total percent agreement was 97% (95% CI, 0.94 - 0.99), with a κ statistic of 0.90 (95% CI, 0.83 - 0.97), demonstrating strong agreement between assays. Overall, a positive percent agreement of 89% (95% CI, 0.76 - 0.96) and a negative percent agreement of 99% (95% CI, 0.97 to 0.99) were observed when OP swabs from asymptomatic patients were tested using the DiaSorin Molecular assay (Table 1).

Details for discordant sample analysis are shown in Table 2. An overall discordance rate of 2.8% was found between the two systems. Five specimens (OH-11, OH-22, OH-27, OH-31, OH-34) that were positive on the Roche Cobas were identified as negative by DiaSorin Molecular. Three out of those five samples (OH-11, OH-27, OH-34)

Table 1

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) between DiaSorin Molecular Simplexa and Roche Cobas using oropharyngeal swab specimens in UVT (n = 253).

Molecular Assay	Roche		($\pm$ 95% CI)[a,b]		
	Positive	Negative	PPA[c]	NPA[d]	Kappa (κ)[e]
DiaSorin Molecular					
Positive	40	2[f]	89%	99%	0.90
Negative	5[g]	206	(0.76-0.96)	(0.97- 0.99)	(0.83-0.97)

[a] $\pm$, upper/lower 95%.

[b] CI, confidence interval.

[c] Positive Percent Agreement (PPA).

[d] Negative Percent Agreement (NPA).

[e] Almost-perfect (>0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal (0.21 to 0.39), or none (0 to 0.20).

[f] Two samples had cycle thresholds (Ct) of 36.3 and 36.5 by the DiaSorin Molecular assay for the ORF1ab target, and were negative for the S gene target.

[g] Five samples had cycle thresholds (Ct) of 37.1, 37.9, 32.4, 33.1, and 33.2 by the Roche assay for the PAN-SARS target, and two of the samples were negative for the ORF1ab target.

had Ct values of 37.1, 32.4, and 33.2 for PAN-SARS, respectively, and were considered presumptive positives on initial testing by the Roche Cobas assay since they were negative for the ORF1ab target. Repeat testing by DiaSorin Molecular was also negative for all samples. Additionally, review of records revealed that two of these samples were from patients with previous SARS-CoV-2 infections which had resolved. Therefore, these samples were categorized as true negatives during discordant resolution. Two additional discordant samples (OH-39 & OH-41) were identified as positive for SARS-CoV-2 by the DiaSorin Molecular assay with Ct values of 36.3 and 36.5 for the ORF1ab target but were negative by the Roche Cobas. Following discordant analysis and retrospective chart review, the DiaSorin Molecular SARS-CoV-2 assay showed an improvement of PPA to 95% (95% CI, 0.84 to 0.99). Furthermore, the total percent agreement of DiaSorin Molecular and Roche increased to 98% (95% CI, 0.95 to 1.0), with a κ statistic of 0.94 (95% CI, 0.88 to 1.0) (Table 2).

3.2. Comparison of cycle threshold (Ct) values obtained from asymptomatic patients

Average Ct value distribution obtained by DiaSorin Molecular Simplexa analysis from oropharyngeal-positive patients was compared with that obtained by Roche Cobas. The corresponding R^2 values indicated a good correlation (R^2= 0.7297), with 95% confidence intervals of the slope (0.85 - 1.04) and intercept (-12.01 - 0.93)

between DiaSorin Molecular Simplexa and the Roche Cobas assays (Fig. 1A). Next, the differences in Ct values were plotted against the average values to generate a Bland-Altman plot. The mean difference was 3.6 Cts, with 95% limits of agreement (LoA) of -2.8 - 10.0 (Fig. 1B). Two samples showed a notable discrepancy in Ct values for both targets suggesting an analytical or pipetting error in one assay. Unfortunately, the specimens was not available for retesting. The overall Ct values of both DiaSorin Molecular Simplexa assay targets (S gene and ORF1ab) were significantly lower than that those from Roche Cobas (P = 0.0001), showing an average of 3.6 $\pm$ 1.0 Ct difference for the tested asymptomatic population (Fig. 1C). Further breakdown analysis per target on each assay shows an average 0.8 $\pm$ 1.3 Ct difference between S gene and ORF1ab targets in the DiaSorin Molecular Assay and 0.8 $\pm$ 1.6 Ct difference between the ORF1ab and Pan-SARS for Roche Cobas (Fig. 1D).

4. Discussion

Numerous molecular assays have been granted EUA by the Food and Drug Administration (FDA), however, all of them have been focused on symptomatic individuals. Testing of the symptomatic population was critical early in the pandemic and it was the core of medical decision-making during that time, especially for the population deemed high-risk. Now that we have moved past this initial stage of the pandemic, we have had to adapt to testing of both symptomatic and asymptomatic individuals, and our testing algorithms and criteria for detecting the novel coronavirus have also shifted. Testing individuals without symptoms is now part of the concentrated effort to curb SARS-CoV-2 transmission within the community; From active infection-finding through mandated population testing based on symptoms to contact tracing after possible exposure, pre-requirement testing before being admitted to the hospital for a procedure (e.g., pregnant women admitted for labor and delivery, patients admitted for elective surgery), or screening to safely return to schools or workplaces. Hence, the use of EUA approved assays should not be limited to the symptomatic population, and it is imperative to adopt new testing practices to curb SARS-CoV-2 transmission. While there are performance-driven publications of many EUA assays for symptomatic testing, data on the accuracy and performance of the existing molecular assays for asymptomatic populations is lacking.

Previously, we were able to compare the Simplexa COVID-19 Direct assay clinical performance to Roche Cobas SARS-CoV-2 using samples from symptomatic patients and made several observations, including limit of detection (LOD) and how each test performed in a

Table 2

Details of discordant sample analysis.

Sample ID	Roche Cobas(ORF 1ab/PAN-SARS)	DiaSorin Molecular (S/ORF1ab)	Comment
OH-11	POS (0/37.1)	NEG	Presumptive POS by Roche Cobas. Patient diagnosed with COVID-19 6 weeks prior. Repeat test needed by employer before return to work.
OH-22	POS (35.44/37.9)	NEG	Patient diagnosed with COVID-19 3 weeks prior. Sample repeated for pre-admission testing for procedure.
OH-27	POS (0/32.4)	NEG	Presumptive POS by Roche Cobas. Patient diagnosed with COVID-19 3 months prior. Repeat test for pre-admission testing.
OH-31	POS (33.05/33.1)	NEG	Incidental finding during pre-admission testing. No previous history of COVID-19. Repeat testing 3 days later was negative.
OH-34	POS (0/33.2)	NEG	Presumptive POS by Roche Cobas. Incidental finding during pre-admission testing. No previous history of COVID-19.
OH-39	**NEG**	POS (0/36.3)	Incidental finding during pre-admission testing. No previous history of COVID-19.
OH-41	**NEG**	POS (0/36.5)	Testing for exposure to person with COVID-19 infection.

[a] Discordant sample results are highlighted in bold.

[b] Ct, Cycle threshold.
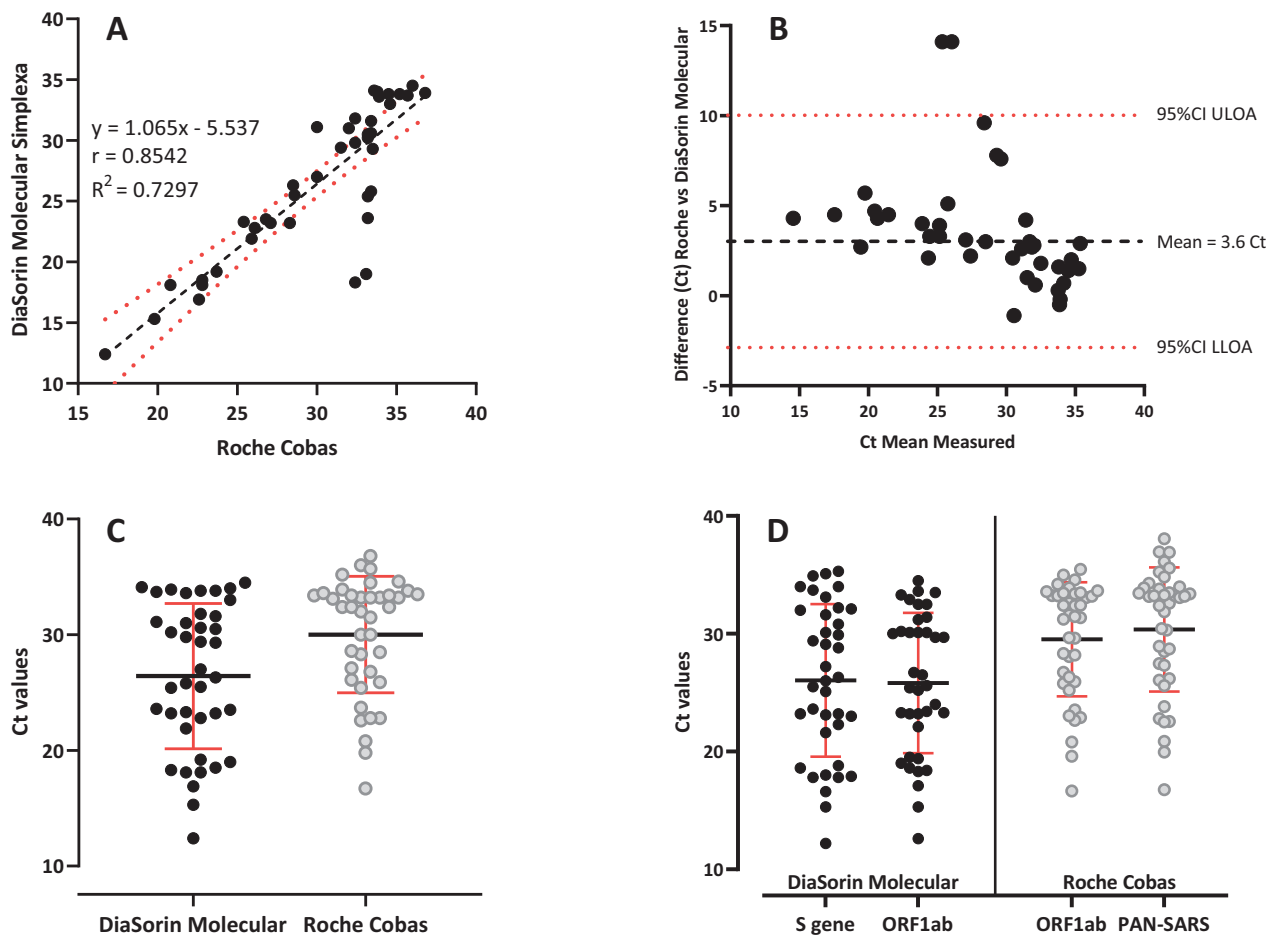
Fig. 1. Comparison of the cycle threshold (Ct) values obtained on the DiaSorin Molecular Simplexa versus Roche Cobas assay for asymptomatic oropharyngeal specimens (*n* = 40). (A) Average Ct value distribution for DiaSorin Molecular Simplexa versus Roche Cobas. The regression line (dashed black line) and 95% confidence intervals (dotted red lines) are displayed. (B) Bland-Altman plot of differences in Ct values versus the average values. The upper and lower 95% limits of agreement (dotted red lines) and mean line (dotted black line) are also shown. (C) Overall Ct values for DiaSorin Molecular and Roche Cobas. Solid red lines indicate 95% limits of agreement and solid black line indicates average Ct. (D) Ct value analysis per target for each assay. Solid red lines indicate 95% limits of agreement and solid black line indicates average Ct. (Color version of figure is available online)

head-to-head clinical comparison (Cradic et al., 2020). With the new measures to prevent the spread of COVID-19 infection, we expanded the comparison to the asymptomatic population. This evaluation is critical as numerous assays are used for testing asymptomatic individuals, and the clinical sensitivity of the assays has not been established thoroughly due to the limited availability of testing resources and the constraints on testing guidelines and clinical recommendations for this population.

Our data suggest that both DiaSorin Molecular and Roche real time-PCR methods yielded comparable results (κ = 0.90), with an overall agreement of 97% (95% CI, 0.94 - 0.99) (Table 1). Of the samples tested, there were a total of seven discordant results between the two assays showing an overall discordance rate of 2.8% between the two systems (Table 2). These discordances may be due to differences in primer sequences, assay limits of detection, or other factors, highlighting the importance of comparing the performance of different testing platforms. Upon further review of the medical chart of those patients where discordance was observed, it was shown that three patients (OH-11, OH-22, OH-27) were previously positive patients who returned for care to our institution seeking a negative test result for medicosocial reasons. These patients were reported as presumptive positive due to the positivity of only the Pan-SARS target by Roche which is not specific for SARS-CoV-2. In addition, these patients reported no symptoms during the time of collection, were at least three weeks from the time of the initial diagnosis, and may not have been infectious at that time during testing. Although

amplification only occurred in the PAN-SARS target, it is unlikely that the samples were SARS-CoV 1 since the virus is not in circulation. Hence, detection of this target is indicative of the current SARS-CoV-2. There is a possibility with these high Ct specimens, that patients are shedding inactive fragments of viral RNA and that the positive test result is not indicative of active viral replication. This has been previously described in patients 10 days post infection with positive RT-PCR samples but negative viral cultures (Owusu et al., 2021). In addition, according to the Roche IFU the Ct values of these samples are higher than the average Ct values at LOD for both assay targets. Another possibility is the amplification of inactive viral RNA fragments in these samples after previous infection. For other respiratory viruses (SARS-CoV, Middle East respiratory syndrome coronavirus, and influenza virus), it is well known that after the immune system neutralizes these viruses, inactivated viral RNA degrades slowly over time, and may still be detected by RT-PCR for months after infection had resolved (Chan et al., 2004; Oh et al., 2016; Peiris et al., 2003; Wang et al., 2018). This highlights the challenges that many labs may have with highly sensitive molecular diagnostic platforms and high Ct value cut-offs that do not accurately reflect clinical relevance for infections like COVID-19. For two additional discordant results (OH-31, OH-34,), the Roche positive results were an incidental finding. The Ct values were high, and both the PAN-SARS and the ORF1ab targets were positive. DiaSorin Molecular detected two additional positives in patient samples that were negative by Roche (OH-39, OH-41), and these two samples had low viral loads as indicated by the Ct

values of the assay. The quantity of viral target in the clinical specimen is near the assay LOD according to the DiaSorin Molecular IFU and because only the ORF1ab target was positive, which does not encode for a structural protein, there is a possibility that infectious viral particles may not have been present in those samples. However, the OH-41 patient was exposed to the virus, and the positive result could represent early phases of viral replication.

Observed Ct values in asymptomatic positive patients covered a wide range indicating that the viral load in these patients is variable (Fig. 1A-1B). Similar ranges of Ct values have been reported for symptomatic patients (Cradic et al., 2020; Danis et al., 2020; Lee et al., 2020; Pan et al., 2020; Ra et al., 2021,). Hence, when performing tests on asymptomatic patients, both clinical and analytical sensitivity is critical as the most sensitive assay would identify individuals who carry and transmit the virus unknowingly.

In the Bland Altman analysis, our data showed the DiaSorin Molecular Simplexa COVID-19 assay Ct values were, on average, 3.6 Cts lower than those of the Roche Cobas SARS CoV-2. All samples, except two, were within the acceptable upper and lower 95% confidence interval. These two samples were not available for repeat testing and investigation of the discrepant Ct values. The mean difference of 3.6 Cts between the assays (Fig. 1B) may be due to the number of variables that can affect assay performance. Assay Ct values depend on multiple factors including sample volume, the gene targets used for the virus detection, assay parameters, fluorescence thresholds, and the cut-off for each assay. For example, the DiaSorin Molecular Simplexa assay requires 50 μl of a non-extracted sample with 10 μl used directly in the amplification reaction, while Roche Cobas utilizes 600 μl of sample volume that is subsequently extracted and used in the amplification reaction. Additionally, each assay has its specific gene targets; DiaSorin Molecular uses the two SARS-CoV-2 specific targets S gene and ORF1ab, while the Roche Cobas design includes a non-specific PAN-SARS and a specific ORF1ab target. The assay Ct cut-off for each assay also differs. DiaSorin Molecular has an assay cut-off of 40 cycles, compared to 45 cycles for Roche Cobas. These data reinforce the idea previously reported by Rhoads et al. (Rhoads et al., 2020) that Ct values from different assays cannot be compared or correlated as significant differences in absolute Ct values can be demonstrated in the same sample with different assays. Also, Ct values can vary significantly between and within methods by as much as 14 cycles, including tests that assess different gene targets for SARS-CoV-2 (Rhoads et al., 2020). These considerations of each platform's unique capabilities and limitations are essential when laboratories examine the possibility of implementing existing assays for asymptomatic testing, and the study design requirements during method comparisons.

For this study, we examined the correlation of the Ct values between assay targets within both assays to evaluate the efficiency of the assay design when used in the asymptomatic population (Fig. 1C-1D). The primer and probe target design must allow for amplification efficiency and specificity, reducing reagent competition, reducing preferential amplification, and allowing for a similar LOD between the two targets. Additionally, with the new SARS-CoV-2 variants circulating within the population, primer and probe target design must be within conservative regions where viral diversity will have the least potential impact (Anon, Lauring and Hodcroft, 2021). The potential impact of missing positive SARS-CoV-2 samples amongst the rise of SARS-CoV-2 variants is reduced by designing assays with multiple specific targets. As part of the DiaSorin Molecular's Simplexa assay algorithm, if one of the specific SARS-CoV-2 targets is detected, the sample is reported as positive. For Roche, both ORF1a and PAN-SARS are required to be detected to obtain positive results. If PAN-SARS target only is detected, the sample is reported as presumptive positive. We observed similar Ct values between the two targets in both assays and confirmed how they complement each other as part of the assay designed (Fig. 1C-1D).

This study has several limitations. Oropharyngeal swabs (OPS) from asymptomatic individuals were collected and may raise the question if OPS provide accurate detection of SARS-CoV-2 in asymptomatic carriers. To date, there is a lack of published data on the accuracy of alternative sample types such as OPS (Zou et al., 2020). It should be noted, CDC guidelines recommend collecting and testing an upper respiratory specimen, with a nasopharyngeal swab (NPS) being the preferred choice for swab-based SARS-CoV-2 testing. When the collection of an NPS is not possible, an oropharyngeal specimen is acceptable. However, these guidelines are for symptomatic patients and do not comment on what sample type is preferred for asymptomatic testing. Previously, we have shown that there is concordance between an OP and NPS specimen for both Roche and DiaSorin Molecular (Cradic et al., 2020). Additionally, a CDC group showed that NPS and OP swabs did not show meaningful differences in SARS-CoV-2 RNA detection in patients with < 7 days post symptom onset supporting OP swabs use when supply chain issues persist (Patel et al., 2020). A second limitation is that this study included samples from patients from a single geographic region, Central Ohio. However, the patient samples spanned the entire range of clinical positives and reflected our overall true positivity rate between 4%-7% during July 15- August 30, 2020 of the COVID-19 outbreak (Anon). Thirdly, the present assay comparison and characterization were made using assays authorized only for symptomatic patients. Although this study has a small sample size, it is clear that patients reported as asymptomatic can have low viral loads that can be detected by assays proven to have high clinical sensitivities. The testing of asymptomatic individuals can be performed after the evaluation of assays currently approved only for symptomatic individuals. Lastly, it is hard to assess if the current asymptomatic samples used in the study are genuinely asymptomatic or merely pre-symptomatic. With the vast range of symptoms across COVID-19 patients, mild symptoms can be missed as some patients may confuse a sore throat or a runny nose for allergies.

In summary, we have evaluated two molecular *in vitro* diagnostic assays for the qualitative detection of SARS-CoV-2 using OP swabs. Our evaluation data suggest that the DiaSorin Molecular and Roche Cobas assays have comparable results and that both can reliably detect SARS-CoV-2 in an asymptomatic population. As we continue in this evolving pandemic and the available vaccines reach the population, testing efforts will continue shifting in the United States. The focus on testing of symptomatic individuals will decrease and move towards asymptomatic testing as a crucial part of the process of monitoring for infection. The lab testing choices will become increasingly complex as many platforms with widely disparate performance characteristics may not be appropriate for all testing populations. The development of guidance and recommendations for asymptomatic screening by data-driven clinical studies is warranted for public health or infection control initiatives to successfully curb viral spread in future epidemics.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SA, KWC; data collection: PO, LF, LL, TP, JS, AD; analysis and interpretation of results: SA, KWC, NW; draft manuscript preparation: SA. All authors reviewed the results and approved the final version of the manuscript.

Declaration of competing interest

The authors report no conflicts of interest relevant to this article.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2021.115513.

References

https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/key-metrics/testing

https://www.cdc.gov/coronavirus/2019-ncov/variants/index.html (Accessed March 12, 2021).

Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6 (accessed March 12, 2021).

Al-Tawfiq JA, Gautret P. Asymptomatic middle east respiratory syndrome coronavirus (MERS-CoV) infection: extent and implications for infection control: a systematic review. Trav Med Infect Dis 2019;27:27–32.

Al-Tawfiq JA. Asymptomatic coronavirus infection: MERS-CoV and SARS-CoV-2 (COVID-19). Travel Med Infect Dis 2020;35: 101608.

Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. JAMA 2020;323:1406–7.

CDC. Overview of Testing for SARS-CoV-2 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html (accessed March 12, 2021).

Chan JFW, Yuan S, Kok KH, Huang L. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020;395: 514e23.

Chan KH, Poon LL, Cheng VC, Guan Y, Hung IF, Kong J, et al. Detection of SARS coronavirus in patients with suspected SARS. Emerg Infect Dis 2004;10:294–9. doi: 10.3201/eid1002.030610 FebPMID15030700; PMCID: PMC3322905.

Cradic K, Lockhart M, Ozbolt P, Fatica L, Landon L, Lieber M, et al. Clinical evaluation and utilization of multiple molecular in vitro diagnostic assays for the detection of SARS-CoV-2. Am J Clin Pathol 2020;154:201–7.

Investigation TeamDanis K, Epaulard O, Benet T, Gaymard A, Campoy S, Botelho-Nevers E, et al. Cluster of coronavirus disease 2019 (COVID-19) in the French Alps, February 2020. Clin Infect Dis 2020;71:825–32.

FDA. In Vitro Diagnostics EUAs. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas (March 12, 2021).

Furuya-Kanamori L, Cox M, Milinovich GJ, Magalhaes RJS, Mackay IM, Yakob L. Heterogeneous and dynamic prevalence of asymptomatic influenza virus infections. Emerg Infect Dis 2016;22:1052–6.

Granados A, Goodall EC, Luinstra K, Smieja M, Mahony J. Comparison of asymptomatic and symptomatic rhinovirus infections in university students: incidence, species diversity, and viral load. Diagn Microbiol Infect Dis 2015;82:292–6.

He D, Zhao S, Lin Q, Zhuang L, Cao P, Wang M, et al. The relative transmissibility of asymptomatic COVID-19 infections among close contacts. Int J Infect Dis 2020;94:145–7. doi: 10.1016/j.ijid.2020.04.034.

Public Health – Seattle & King County; CDC COVID-19 Investigation TeamKimball A, Hatfield KM, Arons M, James A, Taylor J, Spicer K, et al. Asymptomatic and presymptomatic SARS-CoV-2 infections in residents of a long-term care skilled nursing facility - king county, Washington, March 2020. MMWR Morb Mortal Wkly Rep 2020;69:377–81.

La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. Reprod Biomed Online 2020;41:483–99.

Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159–74.

Lauring AS, Hodcroft EB. Genetic variants of SARS-CoV-2—what do they mean?. JAMA 2021;325:529–31.

Lee S, Kim T, Lee E, Lee C, Kim H, Rhee H, et al. Clinical course and molecular viral shedding among asymptomatic and symptomatic patients with SARS-CoV-2 infection in a community treatment center in the Republic of Korea. JAMA Intern Med 2020;180:1447–52.

Chinese Pediatric Novel Coronavirus Study TeamLu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARS-CoV-2 infection in children. N Engl J Med 2020;382:1663–5.

McArthur L, Sakthivel D, Ataide R, Chan F, Richards JS, Narh CA. Review of burden, clinical definitions, and management of COVID-19 cases. Am J Trop Med Hyg 2020;103:625–38.

McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012;22:276–82.

Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Euro Surveill 2020;25: 2000180.

Oh MD, Park WB, Choe PG, Choi SJ, Kim JI, Chae J, et al. Viral load kinetics of MERS coronavirus infection. N Engl J Med 2016;375:1303–5.

Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. Ann Intern Med 2020;173:362–7.

Owusu Daniel, Pomeroy Mary A, Lewis Nathaniel M, Wadhwa Ashutosh, Yousaf Anna R, Whitaker Brett, et al. Household transmission study team, persistent SARS-CoV-2 RNA shedding without evidence of infectiousness: a cohort study of individuals with COVID-19. J Infect Dis 2021. doi: 10.1093/infdis/jiab107 jiab107.

Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 2020;20:411–2. doi: 10.1016/S1473-3099(20)30113-4 Epub 2020 Feb 24.

Patel MR, Carroll D, Ussery E, Whitham H, Elkins C, Noble-Wang J, et al. Performance of oropharyngeal swab testing compared to nasopharyngeal swab testing for diagnosis of COVID-19 -United States, January-February 2020 [published online ahead of print, 2020 Jun 16]. Clin Infect Dis 2020. doi: 10.1093/cid/ciaa759 ciaa759.

HKU/UCH SARS Study GroupPeiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 2003;361:1767–72.

Ra SH, Lim JS, Kim G, Kim GUK, Kim MJ, Jung J, et al. Upper respiratory viral load in asymptomatic individuals and mildly symptomatic patients with SARS-CoV-2 infection. Thorax 2021;76:61–3.

Rhoads Daniel, Peaper David R, She Rosemary C, Nolte Frederick S, Wojewoda Christina M, Anderson Neil W, et al. College of American Pathologists (CAP) microbiology committee perspective: caution must be used in interpreting the cycle threshold (Ct) value. Clin Infect Dis 2020;72(10):e685–6.

Rothe C, Schunk M, Sothmann P, Bretzel G, Groeschl G, Wallrauch C, et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med 2020;382: 970e1.

Walsh KA, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect 2020;81:357–71.

CAP-China NetworkWang Y, Guo Q, Yan Z, Zhou D, Zhang W, Zhou S, et al. Factors associated with prolonged viral shedding in patients with avian influenza A(H7N9) virus infection. J Infect Dis 2018;217:1708–17.

WHO. Coronavirus disease (COVID-19) outbreak. : World Health Organization; 2020 https://www.who.int/emergencies/diseases/novel-coronavirus-2019 (accessed March 12, 2021).

Wilder-Smith A, Teleman MD, Heng BH, Earnest A, Ling AE, Leo YS. Asymptomatic SARS coronavirus infection among healthcare workers, Singapore. Emerg Infect Dis 2005;11:1142–5.

Yuen K-S, Ye ZW, Fung S-Y, Chan C-P, Jin D-Y. SARS-CoV-2 and COVID-19: the most important research questions. Cell Biosci 2020;10:40.

Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382:1177–9.