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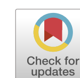
Comparison of diagnostic performance of five molecular assays for detection of SARS-CoV-2



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A B S T R A C T

We compared the performance of the Abbott Real Time SARS-CoV-2 assay (Abbott assay), Aptima™ SARS-CoV-2 assay (Aptima assay), BGI Real-Time SARS-CoV-2 assay (BGI assay), Lyra® SARS-CoV-2 assay (Lyra assay), and DiaSorin Simplexa™ COVID assay for SARS-CoV-2 detection. Residual nasopharyngeal samples (n = 201) submitted for routine SARS-CoV-2 testing by Simplexa assay during June-July 2020 and January 2021 were salvaged. Aliquots were tested on other assays and compared against the CDC 2019-nCoV Real-Time RT-PCR assay. Viral load in positive samples was determined by droplet digital PCR. Among 201 samples, 99 were positive and 102 were negative by the CDC assay. The Aptima and Abbott assays exhibited the highest positive percent agreement (PPA) at 98.9% while the BGI assay demonstrated the lowest PPA of 89.9% with 10 missed detections. Negative percent agreement for all 5 platforms was comparable, ranging from 96.1% to 100%. The performance of all five assays was comparable.
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1. Introduction

Global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a coronavirus disease 2019 (COVID-19) pandemic (Coronavirus disease COVID-19 outbreak, 2020). Molecular methods are currently considered the reference method for SARS-CoV-2 detection (Araj et al., 2020; Hanson et al., 2020; Patel et al., 2020). Many commercial SARS-CoV-2 molecular assays received Emergency Use Authorization (EUA) from the United States Food and Drug Administration (FDA) to meet the diagnostic needs related to this pandemic (Emergency Use Authorization: Emergency Use Authorization EUA information, and list of all current EUAs, 2020). Consequently, clinical laboratories across the United States faced challenges in identifying reliable diagnostic assays for SARS-CoV-2 detection given the limited availability of published performance and comparison data. Additional challenges include supply chain difficulties, which many laboratories have addressed by implementing multiple assays (SARS-CoV-2 molecular testing: summary of recent SARS-CoV-2 molecular testing survey, 2020; Lieberman et al., 2020). Comparative evaluations of analytical performance as well as real world clinical performance of EUA assays are essential to help guide assay selection as laboratories expand testing capacity (Babiker et al., 2020).

Clinical study data comparing several of the molecular assays have recently been published (Basu et al., 2020; Chen et al., 2020; Craney et al., 2020; Creager et al., 2020; Moran et al., 2020; Poljak et al., 2020; Rhoads et al., 2020; Smith et al., 2020; Yip et al., 2020; Zhen et al., 2020). To our knowledge, this is the first study comparing the performance of Lyra SARS-CoV-2 Assay (Lyra assay), and the second large scale clinical study with the BGI Real-Time SARS-CoV-2 assay (BGI assay). Our current study is one of a few that evaluated the clinical diagnostic performance of five or more assays (Garg et al., 2021; Lieberman et al., 2020; Onwuamah et al., 2021). The study objective was to analyze and compare the diagnostic performance of five EUA approved molecular assays, the Abbott Real-time SARS-CoV-2 assay (Abbott assay), Aptima SARS-CoV-2 Assay (Aptima assay), BGI assay, Lyra assay, and DiaSorin Simplexa COVID-19 Direct assay (Simplexa assay), using CDC 2019-nCoV Real-Time RT-PCR assay (CDC assay) as the reference standard on clinical salvage respiratory samples.

2. Methods

2.1. Study design

This prospective salvage sample study included 201 nasopharyngeal samples submitted to the University of Kansas Health System

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Table 1
Molecular *in vitro* diagnostic EUA assay characteristics.

	CDC 2019-nCoV Real-Time RT-PCR assay	Abbott Real-Time SARS-CoV-2 assay	Aptima™ SARS-CoV-2 assay	BGI Real-Time SARS-CoV-2 assay	Lyra® SARS-CoV-2 assay	Simplexa™ COVID-19 Direct assay
Manufacturer	CDC	Abbott	Hologic	BGI	Quidel	Diasorin
Sample type[a]	NP, OP, NP wash/aspirate, nasal aspirate, sputum, BAL	NS, NP, OP, BAL	NP, NS, MTS, OP, NP wash/aspirate, nasal aspirate	NS, NP, MTS, OP, nasal wash/aspirate, BAL	NS, NP, OP	NP, NS, nasal wash/aspirate, BAL
Sample volume (μL)	100	1000	500	180	180	50
Separate extraction	Yes	Yes	No	Yes	Yes	No
Detection system	ABI 7500 Fast Dx	Abbott *m*2000	Panther, Panther Fusion	ABI 7500 Fast, ABI 7500, ABI QS5, Roche LightCycler 480	ABI 7500 Fast Dx, ABI 7500 standard, Roche LightCycler 480, Qiagen Rotor-Gene Q, BioRad CFX96 Touch, ABI QS7	LIAISON MDX
Targets	Dual	Dual	Dual	Single	Single	Dual
Target region of SARS-CoV-2	Nucleocapsid (N) N1, N2	RdRp, N	ORF1ab (2 regions)	ORF1ab	Nonstructural polyprotein (pp1ab)	ORF1ab, S
Analytical sensitivity	31.6−10,000 copies/mL	100 copies/mL	0.01 TCID50/mL	100 copies/mL	250 copies/mL	500 copies/mL
Manufacturer throughput (samples per run)	96	96	1150/24 h	96	96	8
Approximate assay run time[b]	2.5 h	8 h	2.5 h	2.5 h	75 min	90 min
Detection criteria	Target Ct ≤ 40 for any one target	Target Ct ≤ 37	Target RLU ≥ 560	Target Ct ≤ 37	Target Ct ≤ 30	Target Ct ≤ 40

[a] NS = nasal swab; NP = nasopharyngeal swab; OP = oropharyngeal swab; BAL = bronchoalveolar lavage fluid; MTS = midturbinate swab.
[b] Assay run time includes inactivation steps (where applicable) and nucleic acid extraction steps.

(TUKHS) clinical laboratory for routine SAR-CoV-2 testing on the Simplexa COVID-19 Direct assay (DiaSorin Molecular LLC; Cypress, CA) during the months of June/July 2020 and January 2021. Of the 201 specimens analyzed, 100 were positive and 101 were negative on initial testing by Simplexa. Six aliquots of fresh sample were made within 72 hours of the sample collection. Three aliquots were shipped to the Children's Mercy Hospital clinical laboratory on dry ice for testing on three platforms (Aptima assay, CDC assay, and Lyra assay). All aliquots were thawed only once before testing on respective platforms. Testing on Abbott assay and BGI assay were performed at the TUKHS clinical laboratory. Characteristics of all the assays evaluated in the study have been described in Table 1. Test results from each of the five individual assays were compared to CDC reference standard as reported previously (Broughton et al., 2020; Degli-Angeli et al., 2020; Lieberman et al., 2020; Mitchell and George, 2020; Pujadas et al., 2020; Rhoads et al., 2020).The Bio-Rad SARS-CoV-2 ddPCR test was performed on all samples that tested positive by any assay. Ct values obtained from each assay were compared with the quantitative results from ddPCR assay.

This study was approved by the Institutional Review Board of both institutions.

2.2. Specimen collection and storage

Nasopharyngeal swabs (Shenzhen Medico Technology Co.; Shenzhen City, China) were transported in 3 mL of viral transport media (Jinan Babio Biotechnology Co., Ltd.; Shandong, China), stored at 2 to 8°C, and tested with the Simplexa assay. Clinical testing and aliquoting for testing with comparator assays were performed within 72 hours of sample collection. Aliquots were stored at -70°C until further testing.

2.3. Study population

Symptomatic as well as nonsymptomatic patients of both genders were included in the study. Samples were submitted from both inpatient and emergency departments at the TUKHS. Testing location and reason for testing (asymptomatic screen, symptomatic testing) were recorded (Table 2).

2.4. Aptima® SARS-CoV-2 assay (Panther® System)

This assay is a sample-to-answer molecular assay performed on the Panther instrument (Hologic Inc.; Marlborough, MA) that utilizes the combined technologies of target capture, transcription mediated amplification (TMA), and dual kinetic assay (DKA) to amplify two unique regions of the ORF1ab gene of the SARS-CoV-2 viral genome. Briefly, 500 μL of thawed aliquot was transferred to the specimen lysis tube and loaded on the Panther instrument. Based off the total Relative Light Units (RLU) and the kinetic curve type, the assay requires any of the two regions of the *ORF1ab* gene to be detected for reporting a positive sample.

Table 2.
Study patient demographics.

		SARS-CoV-2 positive	SARS-CoV-2 negative	All subjects
N		99	102	201
Age (in years), (range)		53 (18−90)	56 (15−92)	57 (15−92)
Gender, female (%)		47/99 (47.5)	51/102 (50.0)	98/201 (48.8)
Reason for testing	Symptomatic	77/99 (77.8)	51/102 (50.0)	128/201 (63.7)
	Admission screen	22/99 (22.2)	51/102 (50.0)	73/201 (36.3)
Testing location	Inpatient	13/99 (13.1)	28/102 (27.5)	41/201 (20.4)
	Emergency department	86/99 (86.9)	74/102 (72.5)	160/201 (79.6)

2.5. Lyra® SARS-CoV-2 assay

This Real-Time RT-PCR assay is targeted toward SARS-CoV-2 non-structural polyprotein gene (*pp1ab*). Briefly, 180 μL of sample and 20 μL of process control (PRC) were extracted by NucliSENS easyMAG (bioMerieux, Inc; Marcy-l'Étoile, France) and eluted in 50 μL. 5 μL of extract and 15 μL of rehydrated lyophilized master mix containing oligonucleotide primers and fluorophore and quencher-labeled probes was amplified by the Applied Biosystems® 7500 Fast Real-Time PCR System (ABI 7500; ThermoFisher Scientific; Waltham, MA) with specified assay protocol (Table 1). This assay requires detection of the *pp1ab* gene to report a positive sample.

2.6. CDC 2019-nCoV Real-Time RT-PCR assay

This RT-PCR assay targets SARS-CoV-2 virus nucleocapsid N1 and N2 genes and the human RNase P gene. Three separate master mix sets for N1, N2, and RNase P were prepared as per protocol IFU. The PCR reaction was performed using 15 μL of each master mix and 5 μL of extracted sample. Amplification was performed on the Applied Biosystems QuantStudio 5 Real-Time PCR System (QNS-5; Thermo-Fisher Scientific). This assay requires both the N1 and N2 regions of the nucleocapsid gene to be detected to be considered a positive sample.

2.7. Abbott Real-Time SARS-CoV-2 assay

This assay is designed to amplify regions of the RdRp and N genes of SARS-CoV-2. Specimens were heat inactivated at 65°C for 30 minutes prior to testing. At least 1 mL of heat inactivated sample was loaded onto the *m*2000*sp* extraction instrument and amplification and detection were performed on the *m*2000*rt* instrument (Abbott Molecular Inc; Green Oaks, IL). The two SARS-CoV-2 specific probes are labeled with the same fluorophore, so detection of either or both genes is considered a positive result.

2.8. BGI Real-Time SARS-CoV-2 assay

This assay is designed to amplify a single ORF1ab region of the SARS-CoV-2 genome. Specimens were extracted on an MGISP-960RS extraction platform using the MGIEasy Nucleic Acid Extraction Kit with 180 μL of specimen eluted in 30 μL. Amplification and detection was performed on the Applied Biosystems 7500 Real-Time PCR System, using 10 μL of extracted sample RNA with 20 μL of BGI PCR mix, per manufacturer's IFU. Data analysis was performed using the Applied Biosystems 7500 Software (v2.3). Following manual setting of thresholds for target and internal control (β-actin), data were analyzed based on Ct value. Samples were considered positive if internal control Ct was <35 and the target Ct value is <37. Specimens were considered "equivocal" if the SARS-CoV-2 target was detectable at Ct ≥37. For this study, these specimens were re-extracted and retested using the BGI assay as per manufacturer instructions. For the purposes of clinical care, such specimens are retested on an alternative platform.

2.9. DiaSorin Simplexa COVID-19 direct assay

This assay is designed to detect the ORF1ab and S regions of the SARS-CoV-2 genome. Briefly, 50 μL of sample was transferred into the direct amplification disk (DAD) in addition to 50 μL of reaction mix. The sealed DAD was loaded into the LIAISON® MDX instrument (DiaSorin Molecular LLC) and run using the COVID-19 protocol. This assay requires detection of only one of the gene targets to be considered positive.

2.10. Bio-Rad droplet digital PCR

The Bio-Rad SARS-CoV-2 ddPCR test is reverse transcription droplet digital assay for the detection of two regions of the viral nucleocapsid gene (N1 and N2). Amplification was performed using 5.5 μL of the easyMAG extract and 16.5 μL Bio-Rad One-Step RT-ddPCR supermix and loaded on QX200 TM AutoDG Droplet Generator (Bio-Rad) and fractionated into up to 20,000 nanoliter-sized droplets which were collected on a new 96-well plate and heat sealed. One-step reverse transcription and amplification were performed on a C1000 Touch™ Thermal Cycler (Bio-Rad) with 96−deep well reaction module using the following thermal cycling conditions: 50°C for 60 minutes (reverse transcription); 95°C for 10 minutes; and 40 cycles of 94°C for 30 seconds, and 55°C for 60 seconds followed by 98°C for 10 minutes (enzyme deactivation) and 4°C for 30 minutes (droplet stabilization). The plate was then transferred to the QX200™ Droplet Reader (Bio-Rad Laboratories, Inc.; Hercules, CA) to measure the fluorescence intensity of each droplet in two channels (FAM and HEX). Fluorescence data were analyzed using the QuantaSoft droplet reader software (V1.7.4, Bio-Rad Laboratories, Inc.) where reactions containing more than 10,000 droplets were considered suitable for data analysis. High concentrations of viral RNA may cause samples to be reported without any negative droplets. Such samples were diluted (1:100 and 1:1000) and processed a second time for droplet generation and PCR, per manufacturer's protocol.

2.11. Data analysis

The true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) categories were determined based on the CDC reference standard used in the study. Two-by-two data tables were used to determine the positive percent agreement (PPA) and the negative percent agreement (NPA) among all five SARS-CoV-2 diagnostic assays. PPA and NPA estimates along with 95% confidence intervals (CI) presented in Tables 3 were calculated from the site http://vassarstats.net/clin1.html. The Kappa score was calculated for each of the diagnosis assays when compared to the CDC assay outcome. Percentile-based confidence intervals for the Kappa score were calculated using bootstrap estimations (1000 replications). All Kappa score calculations were completed using Stata software (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC).

3. Results

A total of 201 samples were tested. Results from each molecular platform were compared to the CDC reference standard result. Both symptomatic (n = 128) and asymptomatic (n = 73) subjects were included in the study. There was approximately equal distribution of male and female study subjects (Female: n = 98/201, 48.8 %). The median age of the patients included in the study was 57 years (Table 2).

The CDC assay reported 99 samples as positive and 102 as negative for SARS-CoV-2. As compared to CDC assay, Aptima and Abbott assays exhibited the highest PPA at 98.9%; PPA for Lyra and Simplexa assays were 97.9% and 91.9%, respectively. The BGI assay demonstrated the lowest PPA of 89.9% with 10 missed detections. The NPA for all 6 platforms were comparable ranging from 96.1% to 100%. (Table 3). The Aptima assay was determined to have the highest number of false positive (FP) results compared to the CDC reference standard (n = 4). Cohen's kappa values indicated almost perfect agreement (≥0.90) with the reference standard for all five molecular assays.

A total of 106 samples positive by at least one platform were tested by ddPCR to determine viral load. Of the 106 samples, 21 samples were reported as negative on at least one platform and were

Table 3.
Performance characteristics of assays.

Assay	NAAT result	CDC result		PPA (%) (95% CI)	NPA (%) (95% CI)	Kappa (95% CI)[a]
		Positive	Negative			
Lyra	Positive	91	0	91.9 (84.2–96.2)	100 (95.5–100)	0.92 (0.86, 0.97)
	Negative	8	102			
Aptima	Positive	98	4	98.9 (93.7–99.9)	96.1 (89.7–98.7)	0.95 (0.90, 0.99)
	Negative	1	98			
Abbott	Positive	98	3	98.9 (93.7–99.9)	97.1 (91.0–99.2)	0.96 (0.92, 0.99)
	Negative	1	99			
Simplexa	Positive	97	3	97.9 (92.2–99.6)	97.1 (91.0–99.2)	0.95 (0.90, 0.99)
	Negative	2	99			
BGI	Positive	89	0	89.9 (81.8–94.8)	100 (95.4–100)	0.90 (0.83, 0.96)
	Negative	10	102			

[a] Percentile-based confidence intervals from bootstrap estimations.

included for overall discrepant analysis (Table 4). By testing with the CDC assay, 14 of the 21 discordant samples were negative and 7 were positive. The ddPCR result was in agreement with the original CDC result in 18/21 (85.7%) overall discordant samples. The remaining 3 samples were negative by ddPCR (viral load below the 100 copies/ml threshold) and positive by CDC testing. Each of these samples was detected at a high Ct value (or low RLU value) by the CDC assay and most other tested assays and the low viral burden of these samples may account for the negative ddPCR result (Table 4).

Overall viral load in all positive samples ranged between 150 copies/ml to 98.5 million copies/mL for *N1* gene and 162 copies/mL to 93.8 million copies/mL for *N2* gene. Positive samples with the same viral load demonstrated variable Ct values across the different platforms tested. An overall correlation of the Ct values from different assays and the corresponding viral loads as determined by ddPCR for all 106 samples is demonstrated in Fig. 1A and B.

The BGI assay demonstrated the highest number of false negative results ($n = 10$). Three samples had a Ct value between 37 and 40 on initial testing, interpreted as "equivocal". Repeat testing of these samples on BGI platform yielded negative results. On the BGI assay, five samples required repeat testing after initial results were reported with Ct values ranging between 37.17 and 39.72. These samples were re-extracted and retested using the BGI protocol. All five samples yielded negative results on retesting. For the CDC assay, two samples were negative on N1 target but positive on N2 target. Repeat testing with old and new extract generated positive results on both targets (N1 Ct: 38.12 and N2 Ct: 37.4) for one sample while the second sample was negative for both targets.

The Aptima platform reported 4 FP results, all of which were detected from the runs using uncapped tubes (catalogue no. PRD 06554 and 06660), possibly resulting in carryover contamination. A 0.67% carryover rate has been reported in the manufacturer's IFU. Forty-two percent of samples (84/201) were run in the Aptima capped tube workflow which was switched to the uncapped workflow during the study when capped tubes became unavailable. The RLU values for three of the four positive specimens were 671, 786, and 987, considered to be low positives (cut-off RLU for Aptima assay is 560) (Pham et al., 2020); RLU for the fourth specimen was 1212.

4. Discussion

Multiple commercial SARS-CoV-2 molecular assays are currently marketed under FDA EUA without undergoing the typical rigorous clinical trials for obtaining FDA clearance/approval. Comparative

Table 4
Discrepant sample analysis by ddPCR.

Sample	# Platforms positive	CDC (Ct)			Lyra (Ct)	Abbott (Ct)	Simplexa (Ct)		BGI (Ct)	Hologic (RLU)	ddPCR (cp/mL)		
		Result	N1	N2			S	Orf1ab			N1	N2	Result
1	5	Pos	36.2	36.1	ND	23.2	34.9	35.0	34.7	1114	500	700	Pos
2	5	Pos	30.6	30.2	22.9	18.5	ND	ND	33.7	1181	17000	16800	Pos
3	5	Pos	37.2	36.5	12.6	23.2	34.1	ND	ND	1149	490	600	Pos
4	5	Pos	34.9	37.9	27.4	24.7	32.4	34.4	ND	1211	150	320	Pos
5	5	Pos	35.9	34.6	28.9	25.8	ND	39.4	ND	956	331	335	Pos
6	5	Pos	34.1	34.7	27.8	28.1	34.2	34.5	ND	1184	1060	890	Pos
7	5	Pos	35.1	36.1	ND	24.1	26.2	27.1	36.7	1164	327	512	Pos
8	5	Pos	35.6	34.4	27.2	22.2	34.4	35.1	ND	1197	800	900	Pos
9	5	Pos	12.6	10.5	ND	3.4	9.3	9.9	9.6	1216	3.9×10^6	4×10^6	Pos
10	4	Pos	35.2	35.8	ND	27.1	ND	34.2	ND	1137	1500	1600	Pos
11*	4	Pos	36.3	36.8	ND	18.2	ND	39.3	ND	708	97	0	Neg
12*	4	Pos	36.9	39.9	ND	28.7	34.4	ND	ND	677	80	81	Neg
13*	3	Pos	38.1	37.4	ND	27.8	ND	ND	ND	1001	93	99	Neg
14	3	Neg	ND	ND	ND	27.7	33.7	35.6	ND	987	93	92	Neg
15	2	Pos	38.2	38.9	ND	ND	ND	35.2	ND	519	274	192	Pos
16	1	Neg	ND	ND	ND	ND	37.6	ND	ND	278	0	0	Neg
17	1	Neg	ND	ND	ND	ND	32.4	ND	ND	280	0	0	Neg
18	1	Neg	ND	ND	ND	ND	ND	ND	ND	671	110	79	Neg
19	1	Neg	ND	ND	ND	5.56	ND	ND	ND	284	0	112	Neg
20	1	Neg	ND	ND	ND	ND	ND	ND	ND	1212	0	0	Neg
21	1	Neg	ND	ND	ND	ND	ND	ND	ND	786	0	0	Neg

ND = not detected; Pos = positive; Neg = negative.
* Samples with discordant results between CDC and ddPCR assays
ddPCR result cutoff: negative if both targets are <100 copies/mL and <2 positive droplets.

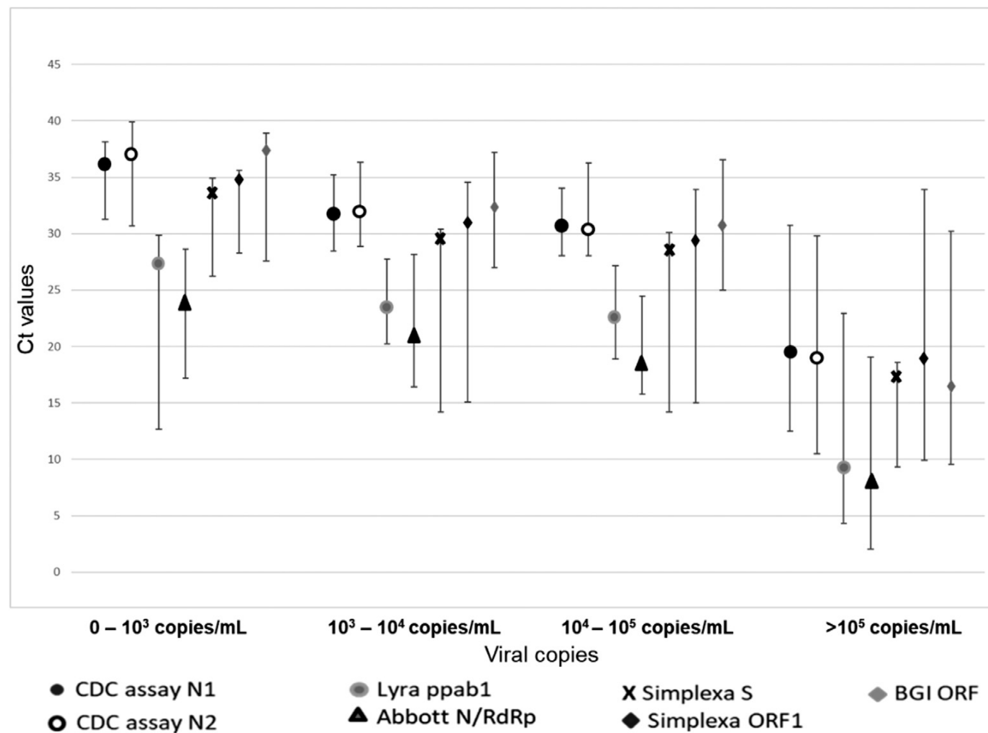

Fig. 1. (A). Correlation of the Ct values (median and range) for assay specific genes with viral load. (B) Correlation of Ct values with viral load of all positive samples on each assay platform.

performance studies for SARS-CoV-2 detection are of importance to the clinical community as they provide real world performance data to help guide testing decisions. This study compared the performance of five SARS-CoV-2 NAATs for 201 NP swab specimens collected from both symptomatic and asymptomatic subjects. Other comparator studies recently evaluated four of the six assays included in our study assay and reported comparable performances (Bordi et al., 2020; Bulterys et al., 2020; Cradic et al., 2020; Creager et al., 2020; Degli-Angeli et al., 2020; Harrington et al., 2020; Lieberman et al., 2020; Moore et al., 2020; Mostafa et al., 2020; Pujadas et al., 2020; Rhoads et al., 2020; Uhteg et al., 2020; Zhen et al., 2020). To our knowledge, this is the first report of relative clinical performance data of the Lyra assay for SARS-CoV-2 detection and one of the few that compares five platforms simultaneously.

The ddPCR is thought to be more sensitive compared to many RT-PCR assays, as there is less dependence on efficiency of PCR amplification due to end point measurement of nucleic acid quantitation. Additionally, ddPCR is less affected by reaction inhibitors due to microdilution in each droplet (Taylor et al., 2017). The ddPCR assay results are reported by gene copy, an absolute quantification, rather than Ct value, which varies significantly among different NAAT platforms (Binnicker, 2020). Ct value varied among different NAATs evaluated in this study, which may be due to protocol differences (e.g., input volume), differences in extraction method (e.g., sample-to-answer vs full extraction), amplification technology (e.g., TMA vs RT-PCR), differences in target region of amplification, sampling error (especially for specimens with low viral load), and assay design variability (e.g., florescence data capture starts after several amplification cycles, as is the case for the Abbott and Lyra assays) (Table 2). The Ct variability across different platforms for samples with the same viral load as demonstrated in this study (Fig. 1A and B) supports the determination that Ct values should be interpreted with caution (Binnicker, 2020; Binnicker, 2020). If required, absolute quantification of viral copies by ddPCR may be a more reliable method of determining viral burden. However, ddPCR missed detection of three true positive samples with low viral loads and corresponding high Ct values on other platforms, demonstrating a limitation that viral loads below the limit of detection of the assay (<100 copies/mL) would go undetected by ddPCR.

Few limitations of our study require consideration. Samples were selected based on the Simplexa assay results and repeat testing after specimen freeze-thaw was not performed on the Simplexa platform, unlike comparable platforms. Theoretically, freeze-thaw may lead to nucleic acid degradation or, alternatively, may remove nonspecific inhibition, both of which may influence detection rate; nonetheless, all assays were comparable in detection rate. Additionally, this study included predominantly samples collected from adult population with only one sample from a patient <18 years old. Therefore, caution is advised while extrapolating study results to a pediatric population. The CDC PCR assay was used as a reference standard method in this study. The absence of true reference standard for SAR-CoV-2 detection is well recognized (Axell-House et al., 2020; Mitchell et al., 2020). Laboratorians have used different approaches for establishing an arbitrary reference standard for diagnostic assay comparison studies such as using composite/consensus reference standard or comparing results to one of the RT-PCR results (Axell-House et al., 2020). We considered the CDC assay as an arbitrary reference method, as it has demonstrated excellent performance characteristics in previous studies (Etievant et al., 2020; Lieberman et al., 2020; Moore et al., 2020; Uhteg et al., 2020). We recognize that a more sensitive assay could alter the study results. We also evaluated results using consensus reference standard, defined as positive result in ≥4 of 6 NAAT assays and consensus negative was a negative result in ≥4 of 6 NAAT assays (data not shown). Results from consensus reference method demonstrated comparable results to CDC reference method; additionally, the CDC assay and consensus reference method were found to have a PPA and NPA of 100% and 99%, respectively (data not shown). Study strengths include direct comparison of five commonly

used EUA approved NAAT platforms for SARS-CoV-2 detection. The sample pool came from both symptomatic and nonsymptomatic patients.

With the presence of multiple diagnostic assays in the field, the decision to select a SARS-CoV-2 molecular assay depends upon the laboratory setting and infrastructure, resources with respect to staff and cost involved, testing volume, patient population, and indication for testing. Assays detecting only a single gene target pose a theoretical risk for false negative results with the emergence of viral variants, although this was not evaluated in this study. Our study evaluated the performance of five platforms in a controlled study design and our data shows comparable results for SARS-CoV-2 detection across all the platforms tested.

Author contributions statement

Neena Kanwar: Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Roles/ Writing - original draft; Writing - review & editing.

Dithi Banerjee: Data curation; Formal analysis; Methodology; Writing- review & editing.

Anjana Sasidharan: Data curation; Writing - review & editing.

Ayah Abdulhamid: Data curation

Marissa Larson: Data curation

Brian Lee: Data curation; Software and Formal analysis

Rangaraj Selvarangan: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

Rachael M Liesman: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

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Declaration of competing interest

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

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