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Analytic and Clinical Performance of Major Commercial Severe Acute Respiratory Syndrome Coronavirus 2 Molecular Assays in the United States

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KEYWORDS

• COVID-19 • SARS-CoV-2 • Emergency use authorization • Molecular
• Analytical sensitivity • Analytical specificity • Clinical performance

KEY POINTS

- Molecular assays to detect SARS-CoV-2 have been rapidly developed in response to the COVID-19 pandemic.
- Comparing the analytical and clinical performance of major commercial SARS-CoV-2 molecular assays provides an objective means of evaluating accuracy before implementation.
- With rare exceptions, molecular assays for the detection of SARS-CoV-2 offer comparable analytical and clinical performance.
- The lessons learned from the COVID-19 pandemic can be applied to the development and implementation of laboratory diagnostics in future outbreaks of novel infectious diseases.

INTRODUCTION

The global coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in more than 276 million cases worldwide and greater than 51 million cases in the United States

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alone.[1,2] With the rapid spread of the virus, the availability of clinical diagnostics to quickly and accurately detect SARS-CoV-2 has been essential to identify positive cases, manage patient care, and guide state and national response plans. To address the need for widescale testing, diagnostic test manufacturers and clinical laboratories have partnered to develop and implement molecular assays at an unprecedented pace. Increasing the testing capabilities in the United States has been facilitated by the issuance of emergency use authorizations (EUAs) by the U.S. Food and Drug Administration (FDA). Molecular diagnostic tests have been the primary means of diagnosing COVID-19, and at the time of preparing this article, greater than 200 SARS-CoV-2 molecular diagnostic tests have received EUA.[3] However, as the number of commercially available SARS-CoV-2 molecular assays has increased, so has the need to understand the differences between these methods. This review compares the analytical and clinical performance of major SARS-CoV-2 molecular assays available in the United States and suggests future topics for consideration.

OVERVIEW OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 MOLECULAR ASSAYS

Selection of Assays

Commercially available SARS-CoV-2 molecular assays were included in this review if they were (1) listed in the 2021 College of American Pathologists' (CAP) Quality Cross Check: SARS-CoV-2 Molecular Program COV2Q-A Participant Summary, and (2) $\geq$20 participating laboratories were listed as using the method in the CAP summary.[4,5] The assays meeting these criteria are summarized in **Table 1**. The FDA maintains a complete list of individual EUAs for SARS-CoV-2 molecular diagnostic tests on its website.[3] Multiplexed panels were out of scope for this review.

Table 1
Major commercial SARS-CoV-2 molecular assays classified by turnaround time and throughput

Classification	Assay (Manufacturer)
Rapid/POC[a]	ID NOW COVID-19 (Abbott Diagnostics Scarborough, Inc., Scarborough, ME) Xpert Omni SARS-CoV-2 (Cepheid, Sunnyvale, CA) Xpert Xpress SARS-CoV-2 test (Cepheid)
Sample-to-answer[b]	BD SARS-CoV-2 Reagents for BD MAX System (Becton, Dickinson and Company [BD], Franklin Lakes, NJ) BioGX SARS-CoV-2 Reagents for BD MAX System (BD) BioFire COVID-19 Test (BioFire Defense, LLC, Salt Lake City, UT) Simplexa COVID-19 Direct assay (DiaSorin Molecular LLC, Cypress, CA) ePlex SARS-CoV-2 Test (GenMark Diagnostics, Inc., Carlsbad, CA) ARIES SARS-CoV-2 Assay (Luminex Corporation, Austin, TX) TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Inc., Waltham, MA)
High-throughput[c]	Abbott RealTime SARS-CoV-2 assay (Abbott Molecular, Des Plaines, IL) Aptima SARS-CoV-2 assay (Hologic, Inc., Marlborough, MA) Panther Fusion SARS-CoV-2 Assay (Hologic) cobas SARS-CoV-2 (Roche Molecular Systems, Inc., Pleasanton, CA) Amplitude Solution with the TaqPath COVID-19 High-Throughput Combo Kit (Thermo Fisher Scientific)

Abbreviations: POC, point-of-care; TAT, turnaround time.
[a] TAT of $\leq$1 h; often single-sample throughput.
[b] TAT of $\sim$1 to 4 h; throughput of up to several dozen samples/run.
[c] TAT of >3 to 4 h; throughput of greater than 450 samples/run.

Once molecular SARS-CoV-2 assays were identified for inclusion, they were further divided into one of the following 3 categories, similar to those applied by Fung and colleagues: (1) rapid/point-of-care (POC), (2) sample-to-answer, and (3) high-throughput.[6] Rapid/POC assays were those with a turnaround time (TAT) of $\leq$1 hour, the capability to be performed in a setting with a Clinical Laboratory Improvement Amendments (CLIA) Certificate of Waiver, and having a typical throughput of 1 sample/run.[3,7] Sample-to-answer platforms were those with a TAT of approximately 1 to 4 hours and a capacity to run several dozen samples/run. The final category consisted of assays performed using a high-throughput platform with the capacity to run more than 450 samples/day, but a typical TAT of greater than 3 to 4 hours (see **Table 1**).[3]

Molecular Technologies

To date, most SARS-CoV-2 molecular assays have used real-time reverse transcription–polymerase chain reaction (RT-PCR) technology. However, additional molecular technologies including transcription-mediated amplification (TMA), nested PCR, reverse transcription loop-mediated isothermal amplification (RT-LAMP), or RT-PCR with electrochemical detection have also been developed (**Table 2**).[3]

Molecular Targets

Molecular assays for the detection of SARS-CoV-2 often include greater than 1 gene target. Common targets include the RNA-dependent RNA polymerase (RdRp), nucleocapsid phosphoprotein (N), spike glycoprotein (S), small envelope protein (E), and open reading frame (ORF) genes. Of the assays included in this review, 6 target a single gene, whereas the remainder target $\geq$2 genes (see **Table 2**).[8–23]

Acceptable Specimen Types

Nasopharyngeal (NP) swabs in viral transport media or phosphate-buffered saline have been considered the gold-standard specimen type throughout the COVID-19 pandemic, and NP swabs are considered acceptable for all assays included in this review. In addition to NP swabs, many assays allow for other upper respiratory swab specimens to be tested, including oropharyngeal, nasal, and midturbinate swabs. A full list of acceptable specimen types are included in **Table 2**.[9,16,24–37]

ANALYTICAL PERFORMANCE

Analytical Sensitivity

Limit of detection

The analytical sensitivity (ie, limit of detection [LoD]) of a molecular assay is the lowest concentration of a target that can be detected in at least 19 (95%) of 20 replicates, as defined by the FDA Molecular Diagnostic Template for Commercial Manufacturers.[38] The manufacturers' established LoDs of major commercial SARS-CoV-2 molecular assays are summarized in **Table 3**. Although it is not possible to directly compare LoDs across all SARS-CoV-2 tests because of varying reporting units (eg, copies/mL vs genomic equivalents/mL), the analytical sensitivity varies across commercially available tests. Among assays with analytical sensitivity reported in copies/mL, the manufacturer's established LoD ranges from ~30 copies/mL (cobas SARS-CoV-2) to 750–1000 copies/mL (ePlex SARS-CoV-2).[9,11,13,14,16,17,21,39,40]

Several groups have evaluated these methods and performed independent studies to confirm the LoD against the manufacturers' claims. In many studies, the LoDs were confirmed to be at or below the analytical sensitivity defined by the manufacturer.[6,41–55] Exceptions included the ID NOW COVID-19 and the TaqPath COVID-19

Table 2
Overview of major commercial SARS-CoV-2 molecular assays in the United States[3,8–21,24–37,39,40,84]

Assay	Platforms	Method	Gene Target(s)	Approved Specimen(s)
ID NOW COVID-19	ID NOW Instrument	RT, Isothermal amplification	RdRp	Nasal, NP, throat swabs
Abbott RealTime SARS-CoV-2 assay	Abbott *m*2000 System	Real-time RT-PCR	RdRp, N	NP, OP, nasal swabs; BAL
BD SARS-CoV-2 Reagents for BD MAX System	BD MAX System	Real-time RT-PCR	N1, N2	NP, anterior nasal, MT, OP swabs; NP wash/aspirate, nasal aspirates
BioGX SARS-CoV-2 Reagents for BD MAX System	BD MAX System	Real-time RT-PCR	N1, N2	NP, OP swabs
BioFire COVID-19 Test	FilmArray 2.1 and FilmArray Torch Instrument Systems	RT, Nested multiplex PCR	ORF1ab[a], ORF8	NP, OP, midturbinate, anterior nasal swabs; sputum, endotracheal aspirate, BAL or mini-BAL
Xpert Omni SARS-CoV-2	GeneXpert Omni System	Real-time RT-PCR	E, N2	NP, OP, anterior nasal, MT swabs; nasal wash/aspirate
Xpert Xpress SARS-CoV-2 test	GeneXpert Dx and GeneXpert Infinity Systems	Real-time RT-PCR	E, N2	NP, OP, anterior nasal, MT swabs; nasal wash/aspirate
Simplexa COVID-19 Direct assay	LIAISON MDX	Real-time RT-PCR	ORF1ab, S	NP, anterior nasal swabs; nasal wash/aspirate, BAL
ePlex SARS-CoV-2 Test	ePlex instrument	RT-PCR and electrochemical detection	N[a]	NP swabs
Aptima SARS-CoV-2 assay	Panther and Panther Fusion systems	TMA, chemiluminescent	ORF1ab[a]	NP, OP, anterior nasal, MT swabs; NP wash/aspirate, nasal aspirate
Panther Fusion SARS-CoV-2 Assay	Panther Fusion System	Real-time RT-PCR	ORF1ab[a]	NP, OP, MT, nasal swabs; NP wash/aspirate, nasal wash, BAL
ARIES SARS-CoV-2 Assay	ARIES instrument	RT-PCR	ORF1ab, N	NP swabs
cobas SARS-CoV-2	cobas 6800 and 8800 Systems	Real-time RT-PCR	ORF1 a/b, E	NP, OP, nasal swabs; self-collected anterior nasal (nasal) swabs

Amplitude Solution with the TaqPath COVID-19 High-Throughput Combo Kit	"Authorized real-time PCR instrument"	Real-time RT-PCR	Orf1ab, S, N NP and anterior nasal swabs
TaqPath COVID-19 Combo Kit	"Authorized real-time PCR instrument"	Real-time RT-PCR	Orf1ab, S, N NP, OP, MT, nasal swabs; NP aspirate, BAL, self-collected nasal swabs

Abbreviations: BAL, bronchoalveolar lavage; E, small envelope; MT, midturbinate; N, nucleocapsid phosphoprotein; NP, nasopharyngeal; OP, oropharyngeal; Orf/ORF, open reading frame; PCR, polymerase chain reaction; RdRp, RNA-dependent RNA polymerase; RT, reverse transcription; S, spike glycoprotein; TMA, transcription-mediated amplification.

[a] Targets in 2 regions of a single gene.

Table 3
Analytical and clinical performance of major SARS-CoV-2 molecular assays[3,6,8–21,39–55,57–62,64,65]

	Analytical Performance				Clinical Performance				
	Analytical Sensitivity (LoD)		Analytical Specificity (Cross-Reactivity) (Observed, Y/N)		Positive Percent Agreement		Negative Percent Agreement		
Assay	Claimed	Observed	Claimed	Observed	Claimed	Observed	Claimed	Observed	Reference(s)
ID NOW COVID-19	125 GE/mL	262–20,000 copies/mL	N	NA	100%	48%–94%	100%	98.4%–100%	Abbott; Cradic et al,[57] 2020; Dinnes et al,[58] 2020; Fung et al,[6] 2020; Lee & Song,[65] 2021; Lephart, et al,[41] 2021; Mitchell & George,[59] 2020; Rhoads et al,[60] 2020; Zhen et al, [42] 2020
Abbott RealTime SARS-CoV-2 assay	100 copies/mL	32–53 copies/mL	N	N	100%	93%–96%	100%	100%	Degli-Angeli et al,[43] 2020; Fung et al,[6] 2020; Lephart et al,[41] 2021
BD SARS-CoV-2 Reagents for BD MAX System	640 GC/mL	251 copies/mL	N	NA	100%	100%	97%	96.7%	Yanson et al,[44] 2021
BioGX SARS-CoV-2 Reagents for BD MAX System	40 GE/mL	NA	N	NA	100%	NA	100%	NA	NA
BioFire COVID-19 Test	330 GC/mL	125–165 copies/mL 500 GE/mL	N	NA	90%–100%[a]	98.7%–100%	100%	100%	Eckbo et al, [46] 2021; Smith et al, [45] 2020
Xpert Omni SARS-CoV-2	400 copies/mL	NA	Y[b]	NA	100%	NA	100%	NA	NA
Xpert Xpress SARS-CoV-2 test	0.0200 PFU/mL	0.01 PFU/mL 8.26–100 copies/mL	Y[b]	Y[c]	97.8%	98.3%–100%	95.6%	95.8%–100%	Dinnes, et al,[58] 2020; Lephart et al,[41] 2021; Loeffelholz, et al,[47] 2020; Wolters et al, [48] 2020; Zhen et al, [42] 2020
Simplexa COVID-19 Direct assay	500 copies/mL[d]	39 ± 23–521 copies/mL	Y[e]	N	96.7%–100%[f]	88%–100%	100%	95.5%–100%	Bordi et al, [50] 2020; Cradic, et al, [57] 2020; Fung et al, [6] 2020; Lephart, et al, [41] 2021; Rhoads et al, [60]2020; Zhen et al, [49] 2020; Zhen et al, [42] 2020

ePlex SARS-CoV-2 Test	750–1000 copies/mL[g]	100–1000 copies/mL	Y[h]	NA	94.4%	91.4%–100%	100%	100%	Fung, et al.[6] 2020; Uhteg et al,[51] 2020; Zhen et al,[49] 2020; Zhen et al,[42] 2020
Aptima SARS-CoV-2 assay	0.01 $TCID_{50}$/mL	0.01–0.003 $TCID_{50}$/mL 62.5–612 copies/mL 500 GE/mL	N	N	100%	94.7%–100%	98.2%	98.7%–100%	Pham et al,[52] 2020; Schneider et al,[53] 2021; Smith et al,[45] 2020; Yanson et al,[44] 2021
Panther Fusion SARS-CoV-2 Assay	0.01 $TCID_{50}$/mL	62.5–100 copies/mL 1000 GE/mL	N	NA	100%	98.7%–100%	100%	96%–100%	Fung et al,[6] 2020; Smith et al,[45] 2020; Zhen et al,[49] 2020
ARIES SARS-CoV-2 Assay	180,000 NDU/mL	1000–10,000 copies/reaction range	N	NA	100%[i]	26.7%–100%	100%	100%	Lee et al,[62] 2021; Tanida et al,[54] 2020
cobas SARS-CoV-2	25–46 copies/mL[j]	$\leq$ 10–298 copies/mL	N	NA	100%	94.2%–100%	100%	90%–100%	Cradic et al,[57] 2020; Fung et al,[6] ; Lee et al,[62] 2021; Pujadas et al,[64] 2020; Yanson et al,[44] 2021
Amplitude Solution with the TaqPath COVID-19 High-Throughput Combo Kit	250 GCE/mL[k]	NA	Y[l]	NA	100%	NA	100%	NA	NA
TaqPath COVID-19 Combo Kit	10 GCE/reaction	767 GC/mL	Y[l]	NA	100%	85.3%–100%	100%	70%–100%	Lee et al,[62] 2021; Matsumura et al,[55] 2021

Abbreviations: GC, genomic copies; GCE, genome copy equivalents; GE, genomic equivalents; LoD, limit of detection; N, no; NA, information not available; NDU, nucleic acid amplification test-detectable units; PFU, plaque-forming unit; $TCID_{50}$, median tissue culture infectious dose; Y, yes.

[a] Varies depending on the method of evaluation (eg, contrived vs clinical samples).

[b] E primers and probes will detect human SARS-CoV.

[c] E primers and probe detected SARS-CoV, resulting in a presumptive positive test result.

[d] Specific to nasopharyngeal swabs.

[e] Primer and/or probe sequence homology with SARS-CoV detected by *in silico* analysis, not observed during laboratory testing.

[f] Varies depending on specimen type.

[g] Varies depending on workflow used (with vs without sample delivery device).

[h] Primer and/or probe sequence homology with SARS-CoV by in silico analysis, also observed in laboratory testing.

[i] Overall PPA (PPA varies for individual gene targets).

[j] Varies depending on the target and method of analysis.

[k] Only confirmed through bridging study.

[l] Primer and/or probe sequence homology for N gene with *Neisseria elongata*. Given low homology with N gene reverse primer and probe, the risk for nonspecific amplification was determined to be low. Primer and/or probe sequence homology was also identified for "different isolates of the same species" (eg, strains of *Bacillus anthracis*), but amplification was deemed unlikely to occur.

Combo Kit, both of which demonstrated higher LoDs (262–20,000 and 767 copies/mL, respectively) during independent evaluations.[6,41,42,55] Of the rapid/POC assays, several studies have demonstrated that the Xpert Xpress SARS-CoV-2 test showed superior sensitivity (~10–100 copies/mL) compared with the ID NOW COVID-19 assay (262–20,000 copies/mL).[6,41,42,47,55] Independent studies generally confirmed the claimed analytical sensitivity of sample-to-answer assays, which range from approximately 40 to 1000 copies/mL. In contrast to several other sample-to-answer assays, the ePlex SARS-CoV-2 test has been shown to inconsistently detect samples with lower viral concentrations than the manufacturer's claimed LoD of 750 to 1000 copies/mL. Zhen and colleagues demonstrated that at concentrations of 1000 and 500 copies/mL, a decrease was noted in percent detected from 100% to 70%.[49] All high-throughput assays demonstrated excellent analytical sensitivity, with a study by Fung and colleagues determining the LoD for the cobas SARS-CoV-2 assay to be $\leq$ 10 copies/mL.[6] Yanson and colleagues established a higher LoD for this assay at 298 copies/mL, although details for the lowest concentration tested were unavailable and the LoDs determined for other assays (eg, Aptima SARS-CoV-2 assay) were also significantly higher ($\geq$4 times) than observed in other studies.[44,45,52,53]

Inclusivity

Inclusivity studies can be performed by *in silico* analysis with the purpose of identifying the sequences that will be detected by the assay. Per FDA guidance, assays should detect 100% of SARS-CoV-2 strains, with a required risk assessment describing the potential impact on assay performance should sequences with less than 100% homology be identified during inclusivity studies.[38] Of the reviewed assays, a small number of manufacturers evaluated inclusivity by performing laboratory testing in addition to *in silico* analysis. Manufacturers claimed 86.4% to 100% alignment of oligonucleotide primer and probe sequences with SARS-CoV-2 sequences available in public databases, such as NCBI and GenBank. No manufacturers predicted an impact on the ability of their assay to detect published SARS-CoV-2 strains, including those with less than 100% alignment with available SARS-CoV-2 sequences.[8–21,39,40] It must be noted that reported coverage will vary based on the number of sequences available for comparison at the time the *in silico* analysis is performed. This is especially important as new variants of SARS-CoV-2 emerge.

Analytical Specificity

Cross-reactivity

The analytical specificity of molecular diagnostics can be evaluated through cross-reactivity studies. The purpose of these studies is to ensure that the molecular assay does not react with similar, potentially related pathogens or other organisms that may be present in clinical specimens. The FDA provides a list of recommended organisms to include in cross-reactivity studies by *in silico* analysis and laboratory testing. This includes other members of the family *Coronaviridae* (eg, human coronaviruses 229E, OC43, HKU1, NL63, SARS-CoV, and MERS-CoV) as well as organisms that are likely to be present in respiratory specimens. Recommendations are provided for follow-up studies should significant homology (>80%) with a potential cross-reactive sequence be identified.[38]

Table 3 summarizes the results of cross-reactivity studies and *in silico* analyses performed by manufacturers to ensure analytical specificity. Multiple manufacturers reported the potential for cross-reactivity with coronaviruses known to infect animals (eg, bat and pangolin coronaviruses) as well as SARS-CoV, which is not unexpected because of high genetic homology with SARS-CoV-2. No manufacturers noted cross-

reactivity with MERS-CoV or other organisms likely to be present in respiratory samples.[8,10–21,39,40,56] Independent evaluation of commercial assays has not revealed significant cross-reactivity that would raise concern for false-positive results because of the presence of nonspecific sequences.[43,47,50,52]

CLINICAL PERFORMANCE

Percent Agreement

In addition to analytical studies, the clinical performance must also be evaluated when developing molecular assays for SARS-CoV-2. The FDA recommends calculating positive percent agreement (PPA) in comparison to a high sensitivity EUA RT-PCR test. Furthermore, it is recommended that the comparator assay uses an "internationally recognized standard" or the FDA's SARS-CoV-2 Reference Panel to establish the sensitivity of the assay. Recommendations for assessing the agreement of negative results (ie, negative percent agreement [NPA]) are comparison with an EUA RT-PCR test using prospectively collected samples or "as agreement with expected results if samples were collected from individuals known to be negative for SARS-CoV-2 (eg, collected before December 2019)." The comparator EUA RT-PCR does not need to have identical targets to the assay being evaluated. The acceptance criteria for positive and negative agreement is $\geq$ 95%.[38]

Table 3 summarizes available information on clinical performance, as demonstrated by PPA and NPA between methods. Manufacturer claims for overall PPA ranged from 90% to 100%.[8–21,39,40] The ARIES SARS-CoV-2 assay demonstrated only 25% to 40% agreement for the ORF1ab target, but 100% agreement for the N target; however, only 1 of the 2 targets must be detected for the assay result to be interpreted as positive.[18] During independent evaluations, the observed PPA for most commercial assays was similar to manufacturer claims with the exception of the ID NOW COVID-19 device and ARIES SARS-CoV-2 assay, which both claimed 100% PPA and demonstrated PPA ranging from 48% to 94% and 26.7% to 100% in published studies, respectively.[41,42,54,57–62] Possible explanations for the differences observed with the ID NOW COVID-19 PPA may be variations in the comparator assays and the fact that the manufacturer evaluated PPA at 2 to 5 times the LoD, while the referenced studies may have included samples with lower viral loads.[10] The study that determined the ARIES SARS-CoV-2 assay PPA to be 26.7% was based on comparison with the Xpert Xpress SARS-CoV-2 as the reference method and specifically evaluated weakly positive samples (ie, Ct > 34 in the Xpert Xpress SARS-CoV2 assay). When only strongly positive samples (ie, Ct < 34 for at least one target gene in the Xpert Xpress SARS-CoV2 assay) were included in the analysis, PPA increased to 100%.[54] Manufacturer claims for NPA ranged from 95.6% to 100%.[8–16,18–21,39,40,63] Most observed NPAs were similar to manufacturer claims, with the exception of the TaqPath COVID-19 Combo Kit, for which a single study observed 70% NPA based on consensus of 4 molecular assays.[41–45,47,49,50,53,57,58,61,62,64,65]

Comparison with Clinical Evaluation

Although the "gold standard" for the diagnosis of SARS-CoV-2 infections is molecular testing, there is limited information on the clinical performance of these assays through comparison with clinical findings and radiologic evidence of COVID-19.[66,67] In particular, chest computerized tomography (CT) has been suggested as a complementary diagnostic test for patients with suspected SARS-CoV-2 infection.[68] Studies in which patients underwent both RT-PCR testing and chest CT imaging suggest that chest CT is highly sensitive for the diagnosis of SARS-CoV-2 infection and can identify

likely cases of SARS-CoV-2 that were missed by RT-PCR. Two studies of patients in Wuhan, China, demonstrated 97% sensitivity of chest CT using positive RT-PCR results as the reference standard.[68,69] In another study, an in-depth evaluation of 5 patients with initial negative SARS-CoV-2 RT-PCR was performed and showed that chest CT findings were consistent with a SARS-CoV-2 infection before a positive RT-PCR result.[70] Although studies directly evaluating SARS-CoV-2 RT-PCR results with imaging studies and other clinical/epidemiologic findings are few in number, there are now published data showing that in patients with COVID-19 (ie, as determined by clinical and/or radiology findings), SARS-CoV-2 molecular testing may need to be performed multiple times, or on alternate sample types (eg, bronchoalveolar lavage fluid) to yield a positive result.[66,68,70]

It has also been demonstrated that the sensitivity of commercially available molecular assays may depend on when testing is performed during the course of disease. Theoretically, tests detecting SARS-CoV-2 RNA will have the lowest false-negative rate when the viral load is at its highest. He and colleagues proposed that peak viral loads occur around the time of symptom onset, which is typically 3 to 5 days post-exposure.[66,71] This suggests that rapid/POC tests, such as the Xpert Xpress SARS-CoV-2 and ID NOW COVID-19 tests, are likely to provide the highest negative predictive value when performed in symptomatic patients who are early in their disease course. As the clinical course progresses and viral load decreases, the risk for false-negative results increases and using an assay with the lowest (ie, best) analytical sensitivity becomes increasingly important.

DISCUSSION

Although the rapid development and implementation of molecular assays to detect SARS-CoV-2 has addressed the acute need for diagnostic tools during the COVID-19 pandemic, there are remaining questions to consider. Of particular concern is whether currently available molecular assays will continue to detect emerging SARS-CoV-2 variants. The World Health Organization (WHO) continues to partner with leading institutions and experts to identify and classify emerging SARS-CoV-2 variants. These strains are classified as "variants of concern" (VOC) or "variants of interest" (VOI). According to WHO, VOCs are associated with increased transmissibility and/or virulence, a change in COVID-19 epidemiology or disease presentation or compromise the effectiveness of "public health and social measures or available diagnostics, vaccines, therapeutics." As of December 2021, 5 VOCs have been identified and include lineages B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma), B.1.617.2 (delta), and B.1.1.529 (omicron) all of which have been reported in the United States.[2,72,73] In early 2021, the FDA issued a letter to clinical laboratorians and health care providers warning that SARS-CoV-2 variants may not be detected by molecular tests, potentially resulting in false-negative results. Three EUA molecular tests—of which one was included in our review—were identified as potentially limited in their ability to detect variant strains.[74] According to the FDA letter, the S gene target of the TaqPath COVID-19 Combo Kit may have compromised sensitivity in the presence of the B.1.1.7 (alpha) variant, although both the FDA and Thermo Fisher Scientific, Inc., noted that the overall sensitivity of the test is unlikely to be impacted because of the inclusion of multiple targets.[74,75] Furthermore, the manufacturer theorizes that results suggesting S gene dropout (69–70del) may assist in the identification of samples infected with the alpha or omicron variant.[75–77] Of note is the BA.2 descendant lineage of omicron, which does not display the 69-70del and would therefore not be identified by dropout of the S gene.[78] In addition to mutations in the S gene, mutations in the N gene of the

omicron variant may impact detection in molecular tests employing this target. The molecular tests included in our review were not among those identified by the FDA as expected to fail to detect SARS-CoV-2 omicron.[79] The inclusion of multiple gene targets is advantageous and may facilitate the identification of variant strains. The continued emergence of SARS-CoV-2 variants emphasizes the importance of assay design, and highlights the need for redundancy within the test, either by targeting multiple genes or at least 2 unique regions within the same gene.

It must also be noted that several features of COVID-19, such as the period of viral shedding and window of transmission, are not fully defined.[66] With this in mind, a key aspect to consider when assessing the clinical performance of molecular SARS-CoV-2 assays is whether a positive result indicates an active infection or simply the presence of viral RNA from a resolved infection. A study evaluating hospitalized patients with COVID-19 noted that throat swabs and sputum samples remained positive for 2 and 3 weeks, respectively, despite the resolution of COVID-19 symptoms.[80,81] Furthermore, replication-competent virus was not recovered from these patients beyond day 8 of symptoms, suggesting the period of active viral infection is likely shorter compared with detection of viral RNA.[80] In the future, the development of new assays that can help to discriminate active from past infections should be a focus for test manufacturers. This will be important to ensure proper allocation of limited resources, avoid unnecessary medical costs for patients, and only isolate patients for the period that they represent a risk for ongoing viral transmission.

Lastly, the frequency of false-negative molecular SARS-CoV-2 results requires further study. A study by Green and colleagues evaluated a large cohort of 27,377 SARS-CoV-2 molecular assays from 22,338 patients with testing performed by New York-Presbyterian laboratories and included a review of patients with repeat testing (n = 3432 patients [2413 initial negative results, 802 initial positive results]). Most testing was performed using the Roche SARS-CoV-2 test performed on the cobas 6800, with a smaller proportion performed using the ID NOW, Xpert Xpress, Panther Fusion, and in-house developed assays. In patients with repeat testing, 60 oscillated between positive and negative results, emphasizing the need for judicious interpretation of single-test laboratory results in the context of clinical symptoms.[66]

LIMITATIONS

This review is meant to provide an overview of the analytical and clinical performance of major commercial SARS-CoV-2 molecular assays in the United States. It is not intended to be an exhaustive summary of all available publications and relevant data. The observed analytical sensitivity (ie, LoD) data presented in **Table 3** were not always evaluated and published in the same units and/or using the same specimen type(s) as studies outlined in manufacturers' instructions for use, thereby limiting a direct comparison in many cases. In addition, information on observed analytical specificity (ie, cross-reactivity) was not available for several assays. Finally, the comparator assays used to determine clinical performance (ie, PPA/NPA) varied between studies, which further limits direct comparisons (see **Table 3**).

SUMMARY

Laboratory testing for SARS-CoV-2 has played a key role in the response to the COVID-19 pandemic. The rapid development of molecular assays has been crucial to identify positive cases, limit transmission of the virus, and manage patient care decisions. Overall, commercially available molecular assays for the detection of SARS-CoV-2 have demonstrated comparable performance. However, the sensitivity of these

assays has been shown to vary, especially when performed at different time points during the course of COVID-19 disease and on different specimen types (eg, NP swabs vs oropharyngeal swabs). Although rapid, POC molecular tests may assist in making a timely diagnosis of COVID-19, a negative result may not definitively rule out the disease and follow-up testing using a laboratory-based assay may be required.[42,59,82,83] Future test development should focus on variant detection and discrimination, as well as differentiating active viral infection from persistent detection of viral RNA.

CLINICS CARE POINTS

- Greater than 200 SARS-CoV-2 molecular assays have received emergency use authorization by the U.S. Food and Drug Administration.
- In general, the analytic and clinical performance of commercially available molecular SARS-CoV-2 assays has been shown to be comparable by an independent evaluation of these methods.
- The selection of an appropriate commercial molecular SARS-CoV-2 assay is largely dependent on throughput, turnaround time, and cost considerations.
- The emergence of variant strains of SARS-CoV-2 may impact the performance characteristics of molecular assays, particularly those designed to target a single gene.
- Current SARS-CoV-2 molecular assays are unable to differentiate between active infection and persistent viral nucleic acid, which may lead to unnecessary isolation of non-infectious patients.

DISCLOSURE

M.J. Binnicker is a scientific advisory board member for DiaSorin Molecular and Mammoth Biosciences. M.R. Campbell has nothing to disclose.

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