Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Laboratory evaluation of SARS-CoV-2 in the COVID-19 pandemic

Bijal A. Parikh*, Christopher W. Farnsworth

Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, 63110, United States

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A B S T R A C T

Laboratory evaluation of SARS-CoV-2 involves the detection of viral nucleic acid, viral protein antigens, and the antibody response. Molecular detection of SARS-CoV-2 is the only *diagnostic* test currently available in acutely or recently infected individuals. In contrast, serological testing is typically performed once viral RNA has been cleared and symptoms have resolved. This leads to some confusion among clinicians as to which test to order and when each is appropriate. While SARS-CoV-2 assays can suffer from poor sensitivity, all FDA authorized assays to date are intended to be qualitative. Serological tests have multiple assay formats, detect various classes of immunoglobulins, and have a distinct role in seroprevalence studies; however, the association with long-term protection remains unclear. Both molecular and serological testing for SARS-CoV-2 have complementary roles in patient management, and we highlight the challenges faced by clinicians and laboratorians alike in the evaluation and interpretation of the currently available laboratory assays.

* Corresponding author. 660 South Euclid Ave., Campus Box 8118, St. Louis, MO, 63110, United States.
E-mail addresses: bparikh@wustl.edu (B.A. Parikh), cwfarnsworth@wustl.edu (C.W. Farnsworth).

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Molecular diagnostics for SARS-CoV-2

Background

Coronavirus disease 2019 or COVID-19 is caused by the SARS-CoV-2 virus. SARS-CoV-2 is an enveloped virus with a single-stranded RNA genome belonging to the betacoronavirus genera [1]. Other members of this genera include Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV, 2002-3 strain), and the seasonal coronavirus strains OC43 and HKU1 (Fig. 1). Two other seasonal viruses, 229E and NL63, are members of the alphacoronavirus genera and together these two genera comprise all of the coronaviruses known to infect humans. The distinction between SARS-CoV-2 and the seasonal coronaviruses is important in terms of diagnostics for several reasons. First, since these viruses are highly related, molecular targets need to differentiate between the various pathogens. Second, since antibody production is a hallmark of an appropriate immune response following infection, serological testing also needs to distinguish between commonly circulating seasonal strains versus the pandemic strain. Interestingly, many of the molecular diagnostic assays available today contain targets that do not distinguish between SARS-CoV-2, MERS-CoV, and SARS-CoV; however, with the understanding that SARS-CoV has been eradicated [2] and the outbreaks of MERS-CoV have been geographically and clinically limited [3], these assays are effectively only identifying SARS-CoV-2.

Coronaviruses are members of the Order *Nidovirales*, which comprise the longest single-stranded RNA molecule known for viral pathogens, averaging anywhere from 26 to 33 kilobases in length [4]. A schematic of the genome (Fig. 2) demonstrates that over 70% of viral nucleic acid is dedicated to the gene *ORF1ab*, which encodes the essential replication-transcription complex [5]. The RNA-dependent RNA polymerase (RDRP) derived from *ORF1ab* has high fidelity [6], meaning that the transcriptional error rate is below what is typically observed in RNA viruses, a feature likely necessary to maintain such a long genome, while at the same time reducing the chance for rapid acquisition of mutations.

Molecular testing and the FDA emergency use authorization

In response to a variety of external pressures and the impending community-wide spread of SARS-CoV-2, on February 29, 2020, the FDA authorized local clinical and commercial laboratories to develop and use qualified molecular methods for the diagnostic detection of SARS-CoV-2 [7]. The FDA authorization allowed any clinical or commercial laboratory operating under the Clinical Laboratory Improvements Amendment of 1988 (CLIA '88) to develop and provide clinical testing following review by

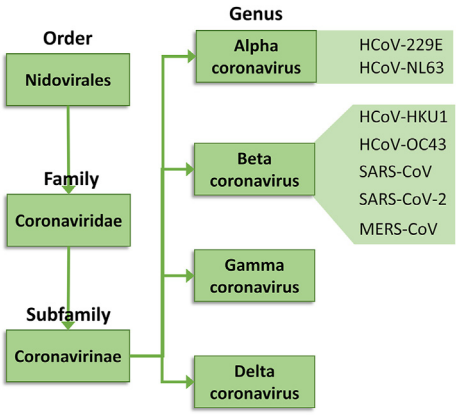

Fig. 1. SARS-CoV-2 belongs to the betacoronavirus genus. The relationship between other human pathogenic coronaviruses is shown. These similarities highlight the need for specific molecular and serologic detection assays.

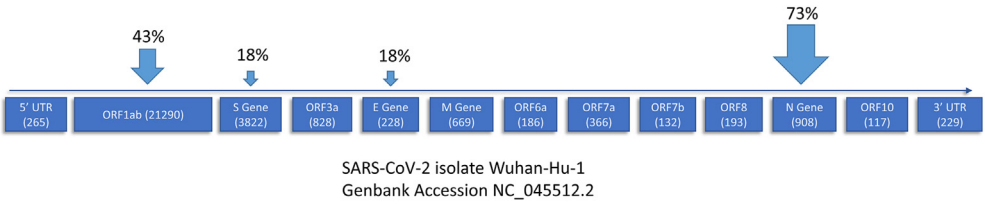

Fig. 2. Genomic organization of SARS-CoV-2 based upon Genbank sequence NC_045512.2. Numbers in parenthesis indicate nucleotide length. Genes targeted by current molecular diagnostic assays are depicted with an arrow, proportional in size to the percentage (above arrow) of assays that target the gene. *ORF1ab* is the largest open reading frame comprising >70% of the genome. Figure not drawn to scale.

the FDA. This review process served to grant the applicant with an Emergency Use Authorization or EUA. The first EUA was issued on Feb 4, 2020 to the CDC and was accessible to clinical laboratories after February 29, 2020.

The EUA process prescribes exactly how an assay must be performed in order to maintain regulatory compliance [8]. In essence, any deviation, including use of alternate thermal cyclers or extractors, negated the authority granted by the EUA. However, provisions were made to be able to "bridge" to related instrumentation if a laboratory could prove through paired sample analysis that the accuracy was not negatively affected. Furthermore, before any laboratory could issue positive or negative results, the first five patients with each result had to be independently verified by either the CDC or the local state laboratory. Until that time, patient results had to report out as "presumptive" positive or negative results. The basis for this requirement stems from the lack of actual patient samples to validate assay performance before the initial surge in COVID-19 patients. Many laboratories simply relied on SARS-CoV-2 RNA "spike-in" experiments, as cultured virus was available only to laboratories equipped with Biosafety Level 3 containment capabilities.

Once the EUA process allowed the entry of more commercial laboratories to clinical testing, a number of assays soon became widely available. The first commercial laboratory with an FDA-approved EUA Kit was ThermoFisher's TaqPath COVID-19 Combo Kit on March 13, 2020. While like the CDC method, this kit required specific instrumentation for extraction and analysis, it broadened the number of laboratories able to participate in testing. This was followed quickly by another manual method, the Quidel Lyra SARS CoV-2 assay on March 17, 2020. Meanwhile, laboratories around the country were developing their own workflows that were not intended to be marketed as test kits for distribution. In March 2020, Labcorp, Quest, the Wadsworth Center, Avellino Labs, and the Yale Clinical Virology Lab were among the first to successfully earn EUA status for their in-house testing. The first two sample-to-answer (no independent extractions required) tests were the Panther Fusion SARS-CoV-2 assay by Hologic and the Abbott RealTime SARS-CoV-2 EUA test, by Abbott Molecular. Finally, testing that could be performed outside CLIA-accredited laboratories in the point-of-care (POC) setting began to appear, further increasing access to diagnostic testing. The first notable entry into the field was Abbott's ID Now. As all of these testing options began to exponentially increase, clinicians were beginning to feel pressure to provide testing to an increasing number of patients who met the epidemiological criteria. However, understanding the challenges and limitations of molecular testing was underestimated by ordering physicians and the clinical laboratory alike.

Molecular detection of SARS-CoV-2

The molecular detection of SARS-CoV-2 requires an initial conversion of RNA into DNA as the substrate for the polymerase chain reaction (PCR)—dependent amplification. A reverse transcriptase or RT enzyme catalyzes this step prior to the PCR. Following the RT step, a fluorophore-dependent system (i.e., Taqman, Molecular Beacons, Scorpion probes, etc.) was used to determine whether viral nucleic acid is present. The strategy initially employed by the CDC assay was to target three different regions of the *N* gene along with a region of the human transcriptome, specifically the *RNase P* gene. This latter

gene served as a specimen adequacy control (SAC), meaning that if *RNase P* was not detected, either the PCR was inhibited by some unknown substance within the patient's sample or that the patient was inadequately sampled. As a result of the limited fluorophores that can be multiplexed, many tests have been developed that replace the SAC in favor of an extraction or processing control (PRC). Specifically, a known (nonhuman) RNA template is spiked into the patient sample prior to extraction and then detected with a unique molecular probe. In all molecular platforms, SARS-CoV-2 (or a control target) is detected if the cycle threshold or CT is crossed prior to the completion of 40 PCR cycles. However, when two targets are used to diagnose SARS-CoV-2, specific instructions must indicate how the discrepancy should be interpreted, as described below. Importantly, all EUA assays for the diagnosis of SARS-CoV-2 are independent of the actual CT value and more simply qualitative in nature. While a great deal of attention has been placed upon understanding the details of molecular detection methods, diagnosis of SARS-CoV-2 RNA is highly comparable to many of the available assays for other clinically important respiratory pathogens.

Targets detected by molecular assays

The first assays with EUA targeted a limited subset of genes on SARS-CoV-2, as specificity from other coronaviruses was important to rule in or out infection with the pandemic strain. The World Health Organization (WHO) and countries outside the US also maintained a list of target sites and some clinical and commercial laboratories adopted these target sequences [9]. However, due to intellectual property protections, the exact target sequence of many commercial assays is currently unknown. A list of SARS-CoV-2 genes targeted by EUA assays is shown in Fig. 2. Notably, the most commonly targeted gene is the *N* gene followed by the long *ORF1ab* gene. The differences in genes targeted and the region of those genes targeted among the individual assays complicate direct comparison of their performance. PCR efficiency, which is determined by the logarithmic increase in amplified products (amplicons), can markedly differ even within a single assay with two unique targets. Often, manufacturers will include highly sensitive gene targets that are less specific for SARS-CoV-2 (and can amplify SARS-CoV and MERS-CoV) along with highly specific but often less sensitive targets, however, as described earlier, given the lack of circulating SARS-CoV and MERS-CoV, during the current pandemic, a presumptive positive is clinically equivalent to a positive result.

Point-of-care molecular testing options

Rheumatologists in private practice have a variety of testing options to select from if they suspect their patient may have COVID-19. Testing that can be performed at the bedside is often preferred for a variety of reasons, including patient satisfaction, rapid turnaround time, and immediate guidance for clinical management. This type of testing parallels the efforts previously achieved with rapid Group A strep and Influenza testing, performed within minutes of sample collection. One of the first offerings in such POC testing included the EUA-approved Abbott ID Now and Cepheid GeneXpert tests for SARS-CoV-2. A laboratorian's visceral reaction to POC testing is often supported by difficulties encountered with the safe and effective deployment of testing outside a controlled laboratory environment. For molecular testing, these issues are “amplified” as even a slight lapse in strict controls can lead to disaster. The foremost issue is environmental contamination and the adherence to necessary mitigation strategies. The Abbot ID Now assay is an open system where the nasopharyngeal (NP) swab is placed directly into the instrument, mixed vigorously, and nucleic acid amplified. The vigorous mixing has a risk of generating aerosols that can lead to contamination of work surfaces, personnel, or the instrument itself. A dedicated biosafety cabinet can be used to minimize these risks, however, this is not common equipment found in physician offices. With these issues aside, the next obstacle is receiving a steady stream of testing equipment and reagents. The high demand for such testing limited and continues to limit access to POC instrumentation. Finally, the sensitivity of the POC assays has been scrutinized and widely debated, especially for the Abbott ID Now [10–13]. If used correctly, though, the sensitivity of the Abbott ID Now was still one of the poorest for all devices on the market with FDA approval (Table 1). For patients with low viral loads that lack the potential to mount sufficient protective responses (e.g., immunocompromised [14]) or those that can potentially spread an undiagnosed

Table 1
EUA assay sensitivity and instructions for SARS-CoV-2 molecular assays.

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Centers for Disease Control and Prevention's (CDC)	CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (CDC)		3.2			18,000	https://www.fda.gov/media/134922/download
Wadsworth Center, New York State Department of Public Health's (CDC)	New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel		25				https://www.fda.gov/media/135847/download
Thermo Fisher Scientific, Inc.	TaqPath COVID-19 Combo Kit		10				https://www.fda.gov/media/136112/download
Laboratory Corporation of America (LabCorp)	COVID-19 RT-PCR test	6250					https://www.fda.gov/media/136151/download
Quidel Corporation	Lyra SARS-CoV-2 Assay			80		6000	https://www.fda.gov/media/136820/download
Quest Diagnostics Infectious Disease, Inc.	Quest SARS-CoV-2 rRT-PCR	136				1800	https://www.fda.gov/media/136231/download
Abbott Molecular	Abbott RealTime SARS-CoV-2 assay			100		5400	https://www.molecular.abbott/sal/9N77-095_SARS-CoV-2_US_EUA_Amp_PI.pdf
Hologic, Inc.	Panther Fusion SARS-CoV-2 assay				0.01	600	https://www.fda.gov/media/136156/download
GenMark Diagnostics, Inc.	ePlex SARS-CoV-2 Test	10,000					https://www.fda.gov/media/136282/download
DiaSorin Molecular LLC	Simplexa COVID-19 Direct assay	242				6000	https://www.fda.gov/media/136286/download
Cepheid	Xpert Xpress SARS-CoV-2 test	250				5400	https://www.fda.gov/media/136314/download
Mesa Biotech Inc.	Accula SARS-CoV-2 test		100				https://www.fda.gov/media/136355/download
BioFire Defense, LLC	BioFire COVID-19 test	330				5400	https://www.fda.gov/media/136353/download
PerkinElmer, Inc.	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	9.3				180	https://www.fda.gov/media/136410/download
Avellino Lab USA, Inc.	AvellinoCoV2 test	55,000				18,000	https://www.fda.gov/media/136453/download
Roche Molecular Systems, Inc. (RMS)	cobas SARS-CoV-2	46				1800	https://www.fda.gov/media/136049/download
Abbott Diagnostics Scarborough, Inc.	ID Now COVID-19			125		300,000	https://www.fda.gov/media/136525/download
NeuMoDx Molecular, Inc.	NeuMoDx SARS-CoV-2 Assay	150					https://www.fda.gov/media/136565/download
Yale New Haven Hospital, Clinical Virology Laboratory	SARS-CoV-2 PCR test	2000					https://www.fda.gov/media/136602/download
Ipsum Diagnostics	COV-19 IDx assay	850					https://www.fda.gov/media/136621/download

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Table 1 (*continued*)

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Becton, Dickinson & Company (BD), BioGx	BioGX SARS-CoV-2 Reagents for BD MAX System			40		1800	https://www.fda.gov/media/136653/download
Diagnostic Molecular Laboratory – Northwestern Medicine	SARS-Cov-2 Assay	100,000					https://www.fda.gov/media/136669/download
Infectious Disease Diagnostics Laboratory - Children's Hospital of Philadelphia	SARS-CoV-2 RT-PCR test	20,000					https://www.fda.gov/media/136656/download
Luminex Corporation	Aries SARS-CoV-2 Assay	330				180,000	https://www.fda.gov/media/136693/download
Co-Diagnostics, Inc.	Logix Smart Coronavirus COVID-19	4290					https://www.fda.gov/media/136687/download
Massachusetts General Hospital	MGH COVID-19 qPCR assay	5000					https://www.fda.gov/media/136699/download
ScienCell Research Laboratories	ScienCell SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit	3000				540	https://www.fda.gov/media/136691/download
Viracor Eurofins Clinical Diagnostics	Coronavirus SARS-CoV-2 RT-PCR assay	73					https://www.fda.gov/media/136740/download
Gnomegen LLC	Gnomegen COVID-19 RT-Digital PCR Detection Kit		8				https://www.fda.gov/media/136738/download
InBios International, Inc	Smart Detect SARS-CoV-2 rRT-PCR Kit	820					https://www.fda.gov/media/136786/download
Becton, Dickinson & Company	BD SARS-CoV-2 Reagents for BD MAX System			40		5400	https://www.fda.gov/media/136816/download
DiaCarta, Inc	QuantiVirus SARS-CoV-2 test	100				600	https://www.fda.gov/media/136809/download
Stanford Health Care Clinical Virology Laboratory	Stanford SARS-CoV-2 assay	1000					https://www.fda.gov/media/136818/download
Atila BioSystems, Inc.	IAMP COVID-19 DETECTION KIT	4000					https://www.fda.gov/media/136870/download
Orig3n, Inc.	Orig3n 2019 Novel Coronavirus (COVID-19) Test	5000					https://www.fda.gov/media/136873/download
Specialty Diagnostic (SDI) Laboratories	SDI SARS-CoV-2 Assay	500					https://www.fda.gov/media/136877/download
University of North Carolina Medical Center	UNC Health SARS-CoV-2 real-time RT-PCR test		17.6			6000	https://www.fda.gov/media/136880/download
Pathology/ Laboratory Medicine Lab of Baptist Hospital Miami	COVID-19 RT-PCR Test	2000				18,000	https://www.fda.gov/media/136944/download

Table 1 (*continued*)

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Integrity Laboratories	SARS-CoV-2 Assay	10,000					https://www.fda.gov/media/136942/download
Infectious Diseases Diagnostics Laboratory (IDDL), Boston Children's Hospital	Childrens-Altona-SARS-CoV-2 Assay	2300				18,000	https://www.fda.gov/media/136971/download
Exact Sciences Laboratories	SARS-CoV-2 Test	125				6030	https://www.fda.gov/media/137095/download
Hackensack University Medical Center (HUMC) Molecular Pathology Laboratory	CDI Enhanced COVID-19 Test	4000				5400	https://www.fda.gov/media/137036/download
CirrusDx Laboratories	CirrusDx SARS-CoV-2 Assay		78			1800	https://www.fda.gov/media/137034/download
Maccura Biotechnology (USA) LLC	SARS-CoV-2 Fluorescent PCR Kit	1000					https://www.fda.gov/media/137026/download
KorvaLabs Inc.	Curative-Korva SARS-Cov-2 Assay	200					https://www.fda.gov/media/137089/download
GenoSensor, LLC	GS COVID-19 RT-PCR Kit	1000					https://www.fda.gov/media/137093/download
Mayo Clinic Laboratories, Rochester, MN	SARS-CoV-2 Molecular Detection Assay	156					https://www.fda.gov/media/137163/download
Altona Diagnostics GmbH	RealStar SARS-CoV02 RT-PCR Kits				0.1		https://www.fda.gov/media/137252/download
Diatherix Eurofins Laboratory	SARS-CoV-2 PCR Test	1000				180,000	https://www.fda.gov/media/137255/download
Southwest Regional PCR Laboratory LLC. dba MicroGen DX	COVID-19 Key	500					https://www.fda.gov/media/137370/download
AIT Laboratories	SARS-CoV-2 Assay	1000					https://www.fda.gov/media/137374/download
Ultimate Dx Laboratory	UDX SARS-CoV-2 Molecular Assay	100					https://www.fda.gov/media/137372/download
Nationwide Children's Hospital	SARS-CoV-2 Assay		12.5				https://www.fda.gov/media/137423/download
Biocerna	SARS-CoV-2 Test	250					https://www.fda.gov/media/137450/download
Rheonix, Inc.	Rheonix COVID-19 MDx Assay	625				1800	https://www.fda.gov/media/137489/download
Altru Diagnostics, Inc.	Altru Dx SARS-CoV-2 RT-PCR assay	625					https://www.fda.gov/media/137546/download
BioFire Diagnostics, LLC	BioFire Respiratory Panel 2.1	300					https://www.fda.gov/media/137583/download
Bio-Rad Laboratories, Inc	Bio-Rad SARS CoV-2-ddPCR Test	625					https://www.fda.gov/media/137579/download
OPTI Medical Systems, Inc.	OPTI SARS-CoV-2 RT PCR Test	900					https://www.fda.gov/media/137739/download
Sherlock BioSciences, Inc.	Sherlock CRISPR SARS-CoV-2 Kit	6750				6000	https://www.fda.gov/media/137746/download

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Table 1 (*continued*)

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Biocollections Worldwide, Inc.	Biocollections Worldwide SARS-Co-V-2 Assay	1000					https://www.fda.gov/media/137897/download
Zymo Research Corporation	Quick SARS-CoV-2rRT-PCR Kit			83			https://www.fda.gov/media/137780/download
Rutgers Clinical Genomics Laboratory at RUCDR Infinite Biologics - Rutgers University	Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2-Assay	200					https://www.fda.gov/media/137782/download
Gnomegen LLC	Gnomegen COVID-19-RT-qPCR Detection Kit		10				https://www.fda.gov/media/137895/download
Abbott Molecular Inc.	Alinity m SARS-CoV-2 assay	100					https://www.fda.gov/media/137979/download
Columbia University Laboratory of Personalized Genomic Medicine	Triplex CII—CoV-1 rRT-PCR Test	280					https://www.fda.gov/media/137983/download
Applied DNA Sciences, Inc.	Linea COVID-19 Assay Kit	1250				2500	https://www.fda.gov/media/138059/download
One Health Laboratories, LLC	SARS-CoV-2 Real-Time RT-PCR-Test	1800					https://www.fda.gov/media/138063/download
Cedars-Sinai Medical Center, Department of Pathology and Laboratory Medicine	SARS-CoV-2-Assay	10,000					https://www.fda.gov/media/138065/download
Hologic, Inc	Aptima SARS-CoV-2 assay				0.01	600	https://www.fda.gov/media/138096/download
Assurance	ASSURANCE SARS-COV-2 PANEL		37			5400	https://www.fda.gov/media/138154/download
Fulgent Therapeutics LLC	Fulgent COVID-19 by RT-PCR Test	5000				3600	https://www.fda.gov/media/138150/download
Color Genomics, Inc.	Color Genomics SARS-CoV-2 RT-LAMP Diagnostic Assay	750				18,000	https://www.fda.gov/media/138249/download
Quidel Corporation	Lyra Direct SARS-CoV-2 Assay	34,000				540,000	https://www.fda.gov/media/138178/download
Seasun Biomaterials, Inc.	AQ-TOP COVID-19 Rapid Detection Kit	7000				6000	https://www.fda.gov/media/138307/download
BioCore Co., Ltd.	BioCore 2019-nCoV Real Time PCR Kit	500				600	https://www.fda.gov/media/138290/download
P23 Labs, LLC.	P23 Labs TaqPath SARS-CoV-2 Assay	10,000					https://www.fda.gov/media/138297/download
Avera Institute for Human Genetics	Avera Institute for Human Genetics SARS-CoV-2 Assay	1600					https://www.fda.gov/media/138332/download
Exact Sciences Laboratories	Exact Sciences SARS-CoV-2 (N gene detection) Test	2600 800					https://www.fda.gov/media/138328/download

Table 1 (*continued*)

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Express Gene LLC (dba Molecular Diagnostics Laboratory)	Express Gene 2019-nCoV RT-PCR Diagnostic Panel						https://www.fda.gov/media/138330/download
dba SpectronRx	Hymon SARS-CoV-2 Test Kit	1200					https://www.fda.gov/media/138345/download
PrivaPath Diagnostics, Inc.	LetsGetChecked Coronavirus (COVID-19) Test				0.01	7200	https://www.fda.gov/media/138406/download
Aspirus Reference Laboratory	Aspirus SARS-CoV-2 rRT Assay	500					https://www.fda.gov/media/138526/download
Gravity Diagnostics, LLC	Gravity Diagnostics COVID-19 Assay	2400				18,000	https://www.fda.gov/media/138530/download
CSI Laboratories	CSI SARS-CoV-2 RT PCR Test	6250					https://www.fda.gov/media/138528/download
Nebraska Medicine Clinical Laboratory	NEcov19 RT-PCR Assay	1000					https://www.fda.gov/media/138625/download
Phosphorus Diagnostics LLC	Phosphorus COVID-19 RT-qPCR Test	5000					https://www.fda.gov/media/138654/download
Euroimmun US Inc.	EUORealTime SARS-CoV-2	150				1800	https://www.fda.gov/media/138761/download
ChromaCode Inc.	HDPCR SARS-CoV-2 Assay	1000				5400	https://www.fda.gov/media/138786/download
Illumina, Inc.	Illumina COVIDSeq Test	1000					https://www.fda.gov/media/138776/download
Warrior Diagnostics, Inc.	Warrior Diagnostics SARS-CoV-2 Assay	150,000					https://www.fda.gov/media/138790/download
Cue Health Inc.	Cue COVID-19 Test	1300					https://www.fda.gov/media/138826/download
Tide Laboratories, LLC	DTPM COVID-19 RT-PCR Test	22,000					https://www.fda.gov/media/138818/download
Cormeum Laboratory Services	Cormeum SARS-CoV-2 Assay	2000					https://www.fda.gov/media/138934/download
RTA Laboratories Biological Products Pharmaceutical and Machinery Industry	Diagnovital SARS-CoV-2 Real-Time PCR Kit	38					https://www.fda.gov/media/138928/download
Kaiser Permanente Mid-Atlantic States	KPMAS COVID-19 Test						https://www.fda.gov/media/139067/download
Applied BioCode, Inc.	BioCode SARS-CoV-2 Assay				0.0172	5400	https://www.fda.gov/media/139049/download
Omnipathology Solutions Medical Corporation	Omni COVID-19 Assay by RT-PCR	1230					https://www.fda.gov/media/139292/download
The Ohio State University Wexner Medical Center	OSUWMC COVID-19 RT-PCR test	250					https://www.fda.gov/media/139288/download
University of Alabama at Birmingham	FRL SARS CoV-2 Test	125					https://www.fda.gov/media/139437/download

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Table 1 (*continued*)

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Fungal Reference Lab							
HealthQuest Esoterics	HealthQuest Esoterics TaqPath SARS-CoV-2 Assay	20				18,000	https://www.fda.gov/media/139434/download
University of Texas MD Anderson Cancer Center, Molecular Diagnostics Laboratory	MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay	1670					https://www.fda.gov/media/139512/download
Diagnostic Solutions Laboratory, LLC	DSL COVID-19 Assay		10				https://www.fda.gov/media/139516/download
PreciGenome LLC	FastPlex Triplex SARS-CoV-2 detection kit (RT-Digital PCR)	571.4					https://www.fda.gov/media/139523/download
Inform Diagnostics, Inc.	Inform Diagnostics SARS-CoV-2 RT-PCR Assay	20,000					https://www.fda.gov/media/139572/download
Acupath Laboratories, Inc.	Acupath COVID-19 Real-Time (RT-PCR) Assay	25,000				18,000	https://www.fda.gov/media/139672/download
LifeHope Labs	LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel			2500			https://www.fda.gov/media/139623/download
Psomagen, Inc.	Psoma COVID-19 RT Test	1000				18,000	https://www.fda.gov/media/139676/download
CENTOGENE US, LLC	CentoFast-SARS-CoV-2 RT-PCR Assay	5000					https://www.fda.gov/media/139725/download
Laboratorio Clinico Toledo	Laboratorio Clinico Toledo SARS-CoV-2 Assay	203					https://www.fda.gov/media/139788/download
Enzo Life Sciences, Inc.	AMPIPROBE SARS-CoV-2 Test System	280				18,000	https://www.fda.gov/media/139828/download
Access Bio, Inc.	CareStart COVID-19 MDx RT-PCR		10			5400	https://www.fda.gov/media/139832/download
Gene By Gene	Gene By Gene SARS-CoV-2 Detection Test	6250					https://www.fda.gov/media/139836/download
Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard	CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay	4000					https://www.fda.gov/media/139858/download
UCSF Health Clinical Laboratories, UCSF Clinical Labs at China Basin	SARS-CoV-2 RNA DETECTR Assay	20,000					https://www.fda.gov/media/139937/download
Boston Medical Center	BMC-CReM COVID-19 Test	10,000					https://www.fda.gov/media/140007/download
		5000					https://www.fda.gov/media/140078/download

Table 1 (*continued*)

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Compass Laboratory Services, LLC	Compass Laboratory Services SARS-CoV2 Assay						
Boston Heart Diagnostics	Boston Heart COVID-19 RT-PCR Test	250				5400	https://www.fda.gov/media/140259/download
Access Genetics, LLC	OraRisk COVID-19 RT-PCR	15,000					https://www.fda.gov/media/140293/download
Helix OpCo LLC (dba Helix)	Helix COVID-19 Test			1000		1800	https://www.fda.gov/media/140420/download
Eli Lilly and Company	Lilly SARS-CoV-2 Assay	1000				18,000	https://www.fda.gov/media/140543/download
Sandia National Laboratories	SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay	6250					https://www.fda.gov/media/140547/download
Clinical Reference Laboratory, Inc.	CRL Rapid Response	250					https://www.fda.gov/media/140661/download
University of California San Diego Health	UCSD RC SARS-CoV-2 Assay	46					https://www.fda.gov/media/140712/download
Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute	Cleveland Clinic SARS-CoV-2 Assay	10,000					https://www.fda.gov/media/140788/download
Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute	Cleveland Clinic SARS-CoV-2 Assay	10,000					https://www.fda.gov/media/140788/download
Ethos Laboratories	Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay				1	5400	https://www.fda.gov/media/140780/download
ISPM Labs, LLC dba Capstone Healthcare	Genus SARS-CoV-2 Assay	40,000					https://www.fda.gov/media/140818/download
Poplar Healthcare	Poplar SARS-CoV-2 TMA Pooling assay				0.01		https://www.fda.gov/media/140792/download
Wren Laboratories LLC	Wren Laboratories COVID-19 PCR Test	15,000					https://www.fda.gov/media/140776/download
Helix OpCo LLC (dba Helix)	Helix COVID-19 NGS Test			125			https://www.fda.gov/media/140917/download
George Washington University Public Health Laboratory	GWU SARS-CoV-2 RT-PCR Test	12,500					https://www.fda.gov/media/140980/download
Alpha Genomix Laboratories	Alpha Genomix TaqPath SARS-CoV-2 Combo Assay	4000					https://www.fda.gov/media/141021/download
Solaris Diagnostics	Solaris Multiplex SARS-CoV-2 Assay	10,000					https://www.fda.gov/media/141016/download
Biomeme, Inc.	Biomeme SARS-CoV-2 Real-Time RT-PCR Test			1800			https://www.fda.gov/media/141052/download
Pro-Lab Diagnostics	Pro-AmpRT SARS-CoV-2 Test			125			https://www.fda.gov/media/141149/download

(*continued on next page*)

Table 1 (*continued*)

Company	Test Name	LOD					IFU
		copies/mL	copies/reaction	GE/mL	$TCID_{50}$/mL	FDA LOD (NDU/mL)	
Yale School of Public Health, Department of Epidemiology of Microbial Diseases	SalivaDirect	6000					https://www.fda.gov/media/141192/download
DxTerity Diagnostics, Inc.	DxTerity SARS-CoV-2 RT-PCR Test	50					https://www.fda.gov/media/141491/download
Guardant Health, Inc.	Guardant-19	125					https://www.fda.gov/media/141487/download
Texas Department of State Health Services, Laboratory Services Section	Texas Department of State Health Services (DSHS) SARS-CoV-2 Assay	20					https://www.fda.gov/media/141496/download
Fluidigm Corporation	Advanta Dx SARS-CoV-2 RT-PCR Assay			6250			https://www.fda.gov/media/141541/download
Cuur Diagnostics	Cuur Diagnostics SARS-CoV-2 Molecular Assay	25,000					https://www.fda.gov/media/141627/download
Patients Choice Laboratories, LLC	PCL SARS-CoV-2 Real-Time RT-PCR Assay	100,000				5400	https://www.fda.gov/media/141665/download
BayCare Laboratories, LLC	BayCare SARS-CoV-2 RT PCR Assay	46					https://www.fda.gov/media/141769/download
MiraDx	MiraDx SARS-CoV-2 RT-PCR assay	4000					https://www.fda.gov/media/141760/download
Mammoth Biosciences, Inc.	SARS-CoV-2 DETECTR Reagent Kit	20,000					https://www.fda.gov/media/141765/download
T2 Biosystems, Inc.	T2SARS-CoV-2 Panel			2000			https://www.fda.gov/media/141755/download
Detectachem Inc.	MobileDetect Bio BCC19 (MD-Bio BCC19) Test Kit	75,000					https://www.fda.gov/media/141791/download

LOD = Limit of Detection; GE = Genome Equivalent; $TCID_{50}$ = Median Tissue Culture Infectious Dose; NDU= Nucleic acid-based amplification tests Detectable Units; IFU= Instructions For Use. Data current as of Sept 17, 2020.

infection to others, such testing may be inadequate. The challenge, of course, is to be able to predict which patients these are. While such low sensitivity has limited implementation of these assays in the hospital setting, it is clear that private practice groups may not be aware of these shortcomings or choose to simply ignore them as often any test is better than no test.

Sensitivity of molecular assays

Until very recently, it was unclear how individual assays performed against each other in terms of sensitivity. Case reports emerged early regarding specific platforms but a systematic analysis has been only recently performed [15]. For this comparison, the US FDA mailed out SARS-CoV-2 reference panels to laboratories who had been approved for clinical testing through the EUA process. As shown in Table 1, stated limits of detection (LODs) found in package inserts ranged in terms of units (copies/mL, copies/reaction, genome equivalents (GE)/mL, or $TCID_{50}$/mL) and value, while the FDA-confirmed LODs provided the first indication of relative sensitivity. The implications for these studies are still not fully understood and whether manufacturers or laboratories will be required to cease testing because of lower sensitivity is also unclear. The clinician is generally unaware of the multiple analytical platforms

their patient could have been tested on and will sometimes ask about the general sensitivity of the assay when a patient they are treating seems to have symptoms consistent with COVID-19 yet test negative. Often, clinicians will request the CT value to better understand the viral load in their patient. It is important to reiterate that there are no *quantitative* tests for SARS-CoV-2 currently approved by the FDA. Unlike blood-borne viral pathogens, including HIV-1 and HCV, it is impossible to standardize the specimen collection for any respiratory viral pathogen. While a high CT value (low viral load) may prompt a clinician to treat a patient less aggressively, the correlation between CT and severity has not been adequately established [16,17]. In fact, recent reports of asymptomatic individuals with high viral loads complicates our ability to predict severity from CT values [18]. Transmissibility, however, may be linked to viral load and studies are underway to investigate this further [19,20]. Until the clinical impact of acting upon CT values is better understood, clinicians are cautioned against using such information to guide patient management.

Clinical and analytical false negatives

There are many reasons why a patient may test negative unexpectedly. While the actual analytical sensitivity is an obvious reason (patient's viral load may be too low to be detected by the chosen platform), other reasons include a more common preanalytical issue that challenges all areas of laboratory medicine (Fig. 3). First, specimen source can affect the outcome of testing. Various testing options available to the clinician for SARS-CoV-2 molecular testing may or may not include all possible specimen types (Table 2) [21]. We have observed patients in which the only positive specimens are retrieved from the lower respiratory tract despite multiple attempts at testing NP swabs [22]. This could reflect the biology of the virus in certain individuals or the clinical disposition of the patient. The emergence of saliva testing as an alternative to the uncomfortable NP swab procedure may impact the rate of false positives as well [23]. Inadequacy of specimen collection [24] cannot always be determined by the assay depending on whether a SAC was included (see Molecular Detection of SARS-CoV-2). Additionally, if patients self-medicate nasal passages with ointments or topical creams, the molecular testing may be inhibited, leading to either an invalid result or possibly a false negative. Other clinical factors impacting pretest probability include whether the patient was symptomatic for COVID-19 and/or had close contact with someone who was infected. The timing of collection relative to the onset of symptoms can also have an effect on test results, as can the time it takes from collection to laboratory testing [25]. Most SARS-CoV-2 molecular tests require refrigeration for no more than 72 h from collection and room-temperature storage for even less time. Beyond these timeframes, if the specimen is not frozen, degradation of the viral RNA can occur. Finally, as supply chain issues have plagued all areas of laboratory testing, collection devices are no exception. The lack of universal transport media (UTM) has necessitated the use of alternative transport media including viral transport media (VTM) containing various antibiotics, normal saline, and phosphate buffered saline (PBS) [26]. The equivalency of these alternative transport media needs to be confirmed by the individual laboratory if not already included in the manufacturer's instructions for use. Even swabs have been in short supply, requiring clinicians to become creative with choice of swabs that are flexible enough to be used in NP sampling, without risking additional harm to patient or being incompatible with molecular testing altogether. In summary, negative results can occur even if the patient has an ongoing SARS-CoV-2 infection, and the laboratory testing component is but one factor in the entire process.

Future state of molecular diagnostic testing

One constant premise laboratories have leaned on is that molecular diagnostics for SARS-CoV-2 changes weekly, if not daily. For example, as regulatory guidance is developed and subsequently modified, the role of the EUA process and FDA oversight has become less clear [27]. While relevant, the complexities of regulatory bodies governing laboratory testing is beyond the scope of the current review, yet heavily influences the availability of quality testing products and suppliers [28]. While it is too early to predict their effectiveness, creative solutions are being considered for a variety of testing issues. These include surveillance testing where individual patient reports are not generated in favor of summary statistics [29]. Additional developments include the ability to pool

patient testing when prevalence is low, thus helping to conserve valuable and often limited testing resources [30]. Finally, as we approach the first influenza season, molecular diagnostics incorporating analytes for both COVID-19 and other respiratory infections are just now entering the market [31,32]. Depending on what the flu season will look like, these may become critical factors in discriminating the types of infections present and triaging to specific therapies. Ultimately, once vaccination is available and broadly dispersed, the role of molecular testing may be less critical, whereas sensitive serological or antigen tests may play a unique role in patient management. In summary, molecular detection of SARS-CoV-2, a single stranded RNA virus, has faced and will continue to be confronted by numerous logistical challenges unseen by the practicing clinician that rapidly impact options for patient testing.

Serology for SARS-CoV-2

Background

In contrast to molecular testing which has had clear diagnostic utility from the onset of the COVID-19 pandemic, the utility of serological assays has been more convoluted [33,34]. This is primarily due to the rapid emergence of serological assays, which has outpaced scientific understanding of their clinical utility. The rapid proliferation of serological assays for SARS-CoV-2 was due at least in part to the FDA's initial decision to not require EUA for their distribution. The reasoning behind this was that these assays were not meant to be diagnostic and that the assays would be used primarily by high-complexity laboratories [35]. However, many of the assays distributed were from relatively unknown vendors using lateral flow devices that resemble pregnancy tests, many of which possessed poor performance characteristics [36]. However, potential for widespread misuse was perpetuated by calls from several entities including the White House Coronavirus Task Force [37] and from health policy experts in high-profile journals [38] arguing for widespread serological testing for SARS-CoV-2 to demonstrate immunity and allow for return to work, etc. However, other major entities including the CDC, WHO, Infectious Diseases Society of America (IDSA), and several laboratory experts have strongly cautioned against rapid and widespread implementation given the unknowns of serological testing including test performance, clinical utility, association with protection, mechanism of protection, and the duration of protection [33,34,39–41]. As a result, the FDA promptly reversed course on May 4, 2020 requiring all serological assays to have EUA and meet certain performance characteristics [42].

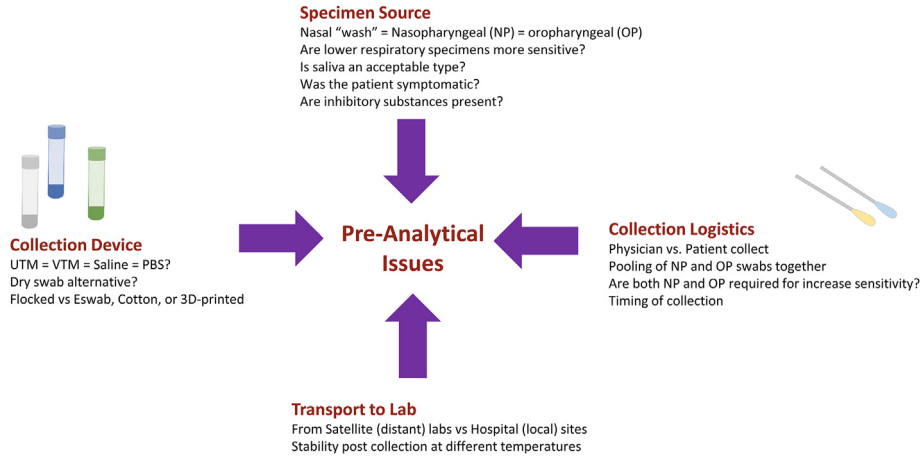

Fig. 3. Common pre-analytical issues faced by clinicians and the diagnostic lab stem from supply chain shortages and uncertain clinical case definitions of COVID-19.

Table 2
EUA assay targets and specimen types for SARS-CoV-2 molecular assays.

Company	Test Name	Format	Date	Gene Target					Specimen Type				
				N	*ORF1ab*	*S*	*E*	Other	*Nasopharyngeal (NP)*	*Oropharyngeal (OP)*	*Upper Respiratory (URT), Other*	*Lower Respiratory (LRT)*	*Saliva*
Centers for Disease Control and Prevention's (CDC)	CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (CDC)	Kit	4-Feb-20	X					X	X	X	X	
Wadsworth Center, New York State Department of Public Health's (CDC)	New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel	Lab	29-Feb-20	X					X	X		X	
Thermo Fisher Scientific, Inc.	TaqPath COVID-19 Combo Kit	Kit	13-Mar-20	X	X	X			X	X	X	X	
Laboratory Corporation of America (LabCorp)	COVID-19 RT-PCR test	Lab	16-Mar-20	X					X	X	X	X	
Quidel Corporation	Lyra SARS-CoV-2 Assay	Kit	17-Mar-20		X				X	X	X		
Quest Diagnostics Infectious Disease, Inc.	Quest SARS-CoV-2 rRT-PCR	Lab	17-Mar-20	X					X	X	X	X	
Abbott Molecular	Abbott RealTime SARS-CoV-2 assay	Kit	18-Mar-20	X	X				X	X	X		
Hologic, Inc.	Panther Fusion SARS-CoV-2 assay	Kit	18-Mar-20	X	X				X	X			
GenMark Diagnostics, Inc.	ePlex SARS-CoV-2 Test	Kit	19-Mar-20	Not specified					X				
DiaSorin Molecular LLC	Simplexa COVID-19 Direct assay	Kit	19-Mar-20		X	X			X		X	X	
Cepheid	Xpert Xpress SARS-CoV-2 test	Kit	20-Mar-20	X			X		X	X	X	X	
Mesa Biotech Inc.	Accula SARS-CoV-2 test	Kit	23-Mar-20	X					X	X	X		
BioFire Defense, LLC	BioFire COVID-19 test	Kit	23-Mar-20		X			*ORF8*	X				
PerkinElmer, Inc.	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	Kit	24-Mar-20	X	X				X	X			
Avellino Lab USA, Inc.	AvellinoCoV2 test	Lab	25-Mar-20	X					X	X			
Roche Molecular Systems, Inc. (RMS)	cobas SARS-CoV-2	Kit	25-Mar-20		X		X		X	X	X	X	
Abbott Diagnostics Scarborough, Inc.	ID Now COVID-19	Kit	25-Mar-20		X				X	X	X		
NeuMoDx Molecular, Inc.	NeuMoDx SARS-CoV-2 Assay	Kit	30-Mar-20	X	X				X	X	X	X	

(*continued on next page*)

Table 2 (*continued*)

Company	Test Name	Format	Date	Gene Target					Specimen Type				
				N	*ORF1ab*	*S*	*E*	Other	*Nasopharyngeal (NP)*	*Oropharyngeal (OP)*	*Upper Respiratory (URT), Other*	*Lower Respiratory (LRT)*	*Saliva*
Yale New Haven Hospital, Clinical Virology Laboratory	SARS-CoV-2 PCR test	Lab	31-Mar-20	X					X	X	X	X	X
Ipsum Diagnostics	COV-19 IDx assay	Lab	1-Apr-20	X					X	X			
Becton, Dickinson & Company (BD), BioGx	BioGX SARS-CoV-2 Reagents for BD MAX System	Kit	2-Apr-20	X					X	X			
Diagnostic Molecular Laboratory – Northwestern Medicine	SARS-Cov-2 Assay	Lab	2-Apr-20	X					X	X	X	X	
Infectious Disease Diagnostics Laboratory - Children's Hospital of Philadelphia	SARS-CoV-2 RT-PCR test	Lab	2-Apr-20	X					X	X	X	X	
Luminex Corporation	Aries SARS-CoV-2 Assay	Kit	3-Apr-20	X	X				X				
Co-Diagnostics, Inc.	Logix Smart Coronavirus COVID-19	Kit	3-Apr-20	Not specified					X	X	X	X	
Massachusetts General Hospital	MGH COVID-19 qPCR assay	Lab	3-Apr-20	X					X	X	X	X	
ScienCell Research Laboratories	ScienCell SARS-CoV-2 Coronavirus Real-time RT-PCR (RT-qPCR) Detection Kit	Kit	3-Apr-20	X					X	X	X	X	
Viracor Eurofins Clinical Diagnostics	Coronavirus SARS-CoV-2 RT-PCR assay	Lab	6-Apr-20	X					X	X	X	X	
Gnomegen LLC	Gnomegen COVID-19 RT-Digital PCR Detection Kit	Kit	6-Apr-20	X					X	X	X		
InBios International, Inc	Smart Detect SARS-CoV-2 rRT-PCR Kit	Kit	7-Apr-20	X	X		X		X	X	X		
Becton, Dickinson & Company	BD SARS-CoV-2 Reagents for BD MAX System	Kit	8-Apr-20	X					X	X			
DiaCarta, Inc	QuantiVirus SARS-CoV-2 test	Kit	8-Apr-20	X	X		X		X	X	X	X	
Stanford Health Care Clinical Virology Laboratory	Stanford SARS-CoV-2 assay	Lab	8-Apr-20				X		X	X	X	X	
Atila BioSystems, Inc.	IAMP COVID-19 DETECTION KIT	Kit	10-Apr-20	X	X				X	X	X		

Orig3n, Inc.	Orig3n 2019 Novel Coronavirus (COVID-19) Test	Lab	10-Apr-20	X				X	X	X		
Specialty Diagnostic (SDI) Laboratories	SDI SARS-CoV-2 Assay	Lab	10-Apr-20		X			X	X			
University of North Carolina Medical Center	UNC Health SARS-CoV-2 real-time RT-PCR test	Lab	10-Apr-20				X	X	X	X	X	
Pathology/Laboratory Medicine Lab of Baptist Hospital Miami	COVID-19 RT-PCR Test	Lab	13-Apr-20	X				X	X	X	X	
Integrity Laboratories	SARS-CoV-2 Assay	Lab	13-Apr-20	X				X	X	X		
Infectious Diseases Diagnostics Laboratory (IDDL), Boston Children's Hospital	Childrens-Altona-SARS-CoV-2 Assay	Lab	14-Apr-20			X	X	X	X	X		
Exact Sciences Laboratories	SARS-CoV-2 Test	Lab	14-Apr-20	X	X		X	X	X	X		
Hackensack University Medical Center (HUMC) Molecular Pathology Laboratory	CDI Enhanced COVID-19 Test	Lab	15-Apr-20	X			X	X	X	X		
CirrusDx Laboratories	CirrusDx SARS-CoV-2 Assay	Lab	15-Apr-20	X	X		X	X	X	X	X	
Maccura Biotechnology (USA) LLC	SARS-CoV-2 Fluorescent PCR Kit	Kit	15-Apr-20	X	X		X	X	X	X		
KorvaLabs Inc.	Curative-Korva SARS-Cov-2 Assay	Lab	16-Apr-20	X				X		X		
GenoSensor, LLC	GS COVID-19 RT-PCR Kit	Kit	16-Apr-20	X	X		X	X	X	X		
Mayo Clinic Laboratories, Rochester, MN	SARS-CoV-2 Molecular Detection Assay	Lab	20-Apr-20	X	X			X	X	X	X	
Altona Diagnostics GmbH	RealStar SARS-CoV02 RT-PCR Kits	Kit	22-Apr-20			X	X	X	X	X		
Diatherix Eurofins Laboratory	SARS-CoV-2 PCR Test	Lab	22-Apr-20	Not specified				X	X	X	X	
Southwest Regional PCR Laboratory LLC. dba MicroGen DX	COVID-19 Key	Lab	23-Apr-20	X				X	X	X	X	
AIT Laboratories	SARS-CoV-2 Assay	Lab	24-Apr-20	X	X	X		X	X	X	X	
Ultimate Dx Laboratory	UDX SARS-CoV-2 Molecular Assay	Lab	24-Apr-20		X			X	X	X		
Nationwide Children's Hospital	SARS-CoV-2 Assay	Lab	27-Apr-20	X				X	X	X	X	
Biocerna	SARS-CoV-2 Test	Lab	28-Apr-20	X	X	X		X	X	X	X	
Rheonix, Inc.	Rheonix COVID-19 MDx Assay	Kit	29-Apr-20	X				X	X	X	X	

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Table 2 (*continued*)

Company	Test Name	Format	Date	Gene Target					Specimen Type				
				N	*ORF1ab*	*S*	*E*	Other	*Nasopharyngeal (NP)*	*Oropharyngeal (OP)*	*Upper Respiratory (URT), Other*	*Lower Respiratory (LRT)*	*Saliva*
Altru Diagnostics, Inc.	Altru Dx SARS-CoV-2 RT-PCR assay	Lab	30-Apr-20	X	X	X			X	X	X		
BioFire Diagnostics, LLC	BioFire Respiratory Panel 2.1	Kit	1-May-20			X		*M*	X				
Bio-Rad Laboratories, Inc	Bio-Rad SARS CoV-2-ddPCR Test	Kit	1-May-20	X					X	X	X		
OPTI Medical Systems, Inc.	OPTI SARS-CoV-2 RT PCR Test	Kit	6-May-20	X					X	X	X	X	
Sherlock BioSciences, Inc.	Sherlock CRISPR SARS-CoV-2 Kit	Kit	6-May-20	X	X				X	X	X	X	
Biocollections Worldwide, Inc.	Biocollections Worldwide SARS-Co-V-2 Assay	Lab	7-May-20	X					X	X	X	X	
Zymo Research Corporation	Quick SARS-CoV-2rRT-PCR Kit	Kit	7-May-20	X					X	X	X	X	
Rutgers Clinical Genomics Laboratory at RUCDR Infinite Biologics - Rutgers University	Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2-Assay	Lab	7-May-20	X	X	X			X	X	X	X	X
Gnomegen LLC	Gnomegen COVID-19-RT-qPCR Detection Kit	Kit	8-May-20	X					X		X		
Abbott Molecular Inc.	Alinity m SARS-CoV-2 assay	Kit	11-May-20	X	X				X	X	X	X	
Columbia University Laboratory of Personalized Genomic Medicine	Triplex CII—CoV-1 rRT-PCR Test	Lab	12-May-20	X					X	X	X		
Applied DNA Sciences, Inc.	Linea COVID-19 Assay Kit	Kit	13-May-20			X			X	X	X		
One Health Laboratories, LLC	SARS-CoV-2 Real-Time RT-PCR-Test	Lab	13-May-20		X	X			X	X	X		
Cedars-Sinai Medical Center, Department of Pathology and Laboratory Medicine	SARS-CoV-2-Assay	Lab	13-May-20	X	X	X			X				
Hologic, Inc	Aptima SARS-CoV-2 assay	Kit	14-May-20		X				X	X	X		
Assurance	ASSURANCE SARS-COV-2 PANEL	Lab	15-May-20				X		X	X	X		
Fulgent Therapeutics LLC	Fulgent COVID-19 by RT-PCR Test	Lab	15-May-20	X					X	X	X		
Color Genomics, Inc.		Lab	18-May-20	X	X		X		X	X	X	X	

	Color Genomics SARS-CoV-2 RT-LAMP Diagnostic Assay											
Quidel Corporation	Lyra Direct SARS-CoV-2 Assay	Kit	18-May-20		X			X	X	X		
Seasun Biomaterials, Inc.	AQ-TOP COVID-19 Rapid Detection Kit	Kit	21-May-20		X			X	X	X	X	
BioCore Co., Ltd.	BioCore 2019-nCoV Real Time PCR Kit	Lab	21-May-20	X	X			X	X	X	X	
P23 Labs, LLC.	P23 Labs TaqPath SARS-CoV-2 Assay	Lab	21-May-20	X	X	X		X	X	X	X	X
Avera Institute for Human Genetics	Avera Institute for Human Genetics SARS-CoV-2 Assay	Lab	22-May-20	X				X	X	X		
Exact Sciences Laboratories	Exact Sciences SARS-CoV-2 (N gene detection) Test	Lab	22-May-20	X				X	X	X		
Express Gene LLC (dba Molecular Diagnostics Laboratory)	Express Gene 2019-nCoV RT-PCR Diagnostic Panel	Lab	22-May-20	X	X	X		X	X	X		
dba SpectronRx	Hymon SARS-CoV-2 Test Kit	Kit	22-May-20	X			X	X	X	X	X	
PrivaPath Diagnostics, Inc.	LetsGetChecked Coronavirus (COVID-19) Test	Lab	28-May-20		X					X		
Aspirus Reference Laboratory	Aspirus SARS-CoV-2 rRT Assay	Lab	1-Jun-20	X				X	X	X	X	
Gravity Diagnostics, LLC	Gravity Diagnostics COVID-19 Assay	Lab	1-Jun-20	X				X	X	X	X	
CSI Laboratories	CSI SARS-CoV-2 RT PCR Test	Lab	2-Jun-20	X				X	X	X	X	
Nebraska Medicine Clinical Laboratory	NEcov19 RT-PCR Assay	Lab	4-Jun-20				X	X	X	X	X	
Phosphorus Diagnostics LLC	Phosphorus COVID-19 RT-qPCR Test	Lab	4-Jun-20	X								X
Euroimmun US Inc.	EURORealTime SARS-CoV-2	Kit	8-Jun-20	X	X			X	X	X	X	
ChromaCode Inc.	HDPCR SARS-CoV-2 Assay	Kit	9-Jun-20	X				X	X	X	X	
Illumina, Inc.	Illumina COVIDSeq Test	Kit	9-Jun-20	Not specified				X	X	X	X	
Warrior Diagnostics, Inc.	Warrior Diagnostics SARS-CoV-2 Assay	Lab	9-Jun-20	X				X				
Cue Health Inc.	Cue COVID-19 Test	Kit	10-Jun-20	X						X		
Tide Laboratories, LLC	DTPM COVID-19 RT-PCR Test	Lab	10-Jun-20	X				X	X	X		
Cormeum Laboratory Services	Cormeum SARS-CoV-2 Assay	Lab	12-Jun-20	X	X	X		X				
		Kit	12-Jun-20		X		X	X	X	X	X	

(*continued on next page*)

Table 2 (*continued*)

Company	Test Name	Format	Date	Gene Target					Specimen Type				
				N	*ORF1ab*	*S*	*E*	Other	*Nasopharyngeal (NP)*	*Oropharyngeal (OP)*	*Upper Respiratory (URT), Other*	*Lower Respiratory (LRT)*	*Saliva*
RTA Laboratories Biological Products Pharmaceutical and Machinery Industry	Diagnovital SARS-CoV-2 Real-Time PCR Kit												
Kaiser Permanente Mid-Atlantic States	KPMAS COVID-19 Test	Lab	13-Jun-20		X		X				X		
Applied BioCode, Inc.	BioCode SARS-CoV-2 Assay	Kit	15-Jun-20	X					X	X	X	X	
Omnipathology Solutions Medical Corporation	Omni COVID-19 Assay by RT-PCR	Lab	17-Jun-20	X					X	X	X	X	
The Ohio State University Wexner Medical Center	OSUWMC COVID-19 RT-PCR test	Lab	17-Jun-20	X					X	X	X		
University of Alabama at Birmingham Fungal Reference Lab	FRL SARS CoV-2 Test	Lab	23-Jun-20	X					X	X	X	X	
HealthQuest Esoterics	HealthQuest Esoterics TaqPath SARS-CoV-2 Assay	Lab	23-Jun-20	X	X		X		X	X	X	X	
University of Texas MD Anderson Cancer Center, Molecular Diagnostics Laboratory	MD Anderson High-throughput SARS-CoV-2 RT-PCR Assay	Lab	24-Jun-20	X					X	X	X		
Diagnostic Solutions Laboratory, LLC	DSL COVID-19 Assay	Lab	25-Jun-20	X			X		X	X	X	X	
PreciGenome LLC	FastPlex Triplex SARS-CoV-2 detection kit (RT-Digital PCR)	Kit	25-Jun-20	X	X					X			
Inform Diagnostics, Inc.	Inform Diagnostics SARS-CoV-2 RT-PCR Assay	Lab	26-Jun-20	X					X	X	X	X	
Acupath Laboratories, Inc.	Acupath COVID-19 Real-Time (RT-PCR) Assay	Lab	29-Jun-20	X	X		X		X		X	X	
LifeHope Labs	LifeHope 2019-nCoV Real-Time RT-PCR Diagnostic Panel	Lab	29-Jun-20	X					X	X	X	X	
Psomagen, Inc.	Psoma COVID-19 RT Test	Lab	30-Jun-20	X					X	X	X	X	
CENTOGENE US, LLC	CentoFast-SARS-CoV-2 RT-PCR Assay	Lab	1-Jul-20		X		X			X			
Laboratorio Clinico Toledo	Laboratorio Clinico Toledo SARS-CoV-2 Assay	Lab	6-Jul-20	X			X		X	X	X	X	
Enzo Life Sciences, Inc.	AMPIPROBE SARS-CoV-2 Test System	Kit	7-Jul-20	X					X	X	X		
Access Bio, Inc.	CareStart COVID-19 MDx RT-PCR	Kit	7-Jul-20	X	X				X	X	X	X	

Gene By Gene	Gene By Gene SARS-CoV-2 Detection Test	Lab	7-Jul-20	X				X			X			
Clinical Research Sequencing Platform (CRSP), LLC at the Broad Institute of MIT and Harvard	CRSP SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Assay	Lab	8-Jul-20	X				X		X	X	X		
UCSF Health Clinical Laboratories, UCSF Clinical Labs at China Basin	SARS-CoV-2 RNA DETECTR Assay	Lab	9-Jul-20	X				X		X	X			
Boston Medical Center	BMC-CReM COVID-19 Test	Lab	10-Jul-20	X				X		X	X	X		
Compass Laboratory Services, LLC	Compass Laboratory Services SARS-CoV2 Assay	Lab	13-Jul-20		X			X		X	X	X		
Boston Heart Diagnostics	Boston Heart COVID-19 RT-PCR Test	Lab	16-Jul-20	X	X	X		X		X	X	X		
Access Genetics, LLC	OraRisk COVID-19 RT-PCR	Lab	17-Jul-20		X			X		X				
Helix OpCo LLC (dba Helix)	Helix COVID-19 Test	Lab	23-Jul-20	X	X	X		X		X	X	X		
Eli Lilly and Company	Lilly SARS-CoV-2 Assay	Lab	27-Jul-20	X				X		X	X	X		
Sandia National Laboratories	SNL-NM 2019 nCoV Real-Time RT-PCR Diagnostic Assay	Lab	27-Jul-20	X				X		X	X	X		
Clinical Reference Laboratory, Inc.	CRL Rapid Response	Lab	30-Jul-20		X								X	
University of California San Diego Health	UCSD RC SARS-CoV-2 Assay	Lab	31-Jul-20		X		X	X		X	X	X		
Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute	Cleveland Clinic SARS-CoV-2 Assay	Lab	3-Aug-20		X		X	X		X	X	X		
Cleveland Clinic Robert J. Tomsich Pathology and Laboratory Medicine Institute	Cleveland Clinic SARS-CoV-2 Assay	Lab	3-Aug-20		X		X	X		X	X	X		
Ethos Laboratories	Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay	Lab	3-Aug-20	X	X			X		X	X	X		
ISPM Labs, LLC dba Capstone Healthcare	Genus SARS-CoV-2 Assay	Lab	3-Aug-20	X				X		X				
Poplar Healthcare	Poplar SARS-CoV-2 TMA Pooling assay	Lab	3-Aug-20		X			X		X	X	X		
Wren Laboratories LLC	Wren Laboratories COVID-19 PCR Test	Lab	3-Aug-20	X				X		X	X	X		
Helix OpCo LLC (dba Helix)	Helix COVID-19 NGS Test	Lab	6-Aug-20			X		X		X	X	X		
George Washington University Public Health Laboratory	GWU SARS-CoV-2 RT-PCR Test	Lab	7-Aug-20	X				X		X	X	X		

(*continued on next page*)

Table 2 (*continued*)

Company	Test Name	Format	Date	Gene Target					Specimen Type				
				N	*ORF1ab*	*S*	*E*	Other	*Nasopharyngeal (NP)*	*Oropharyngeal (OP)*	*Upper Respiratory (URT), Other*	*Lower Respiratory (LRT)*	*Saliva*
Alpha Genomix Laboratories	Alpha Genomix TaqPath SARS-CoV-2 Combo Assay	Lab	10-Aug-20	X	X	X			X	X	X		
Solaris Diagnostics	Solaris Multiplex SARS-CoV-2 Assay	Lab	10-Aug-20	X					X	X	X	X	
Biomeme, Inc.	Biomeme SARS-CoV-2 Real-Time RT-PCR Test	Kit	11-Aug-20		X	X			X	X	X		
Pro-Lab Diagnostics	Pro-AmpRT SARS-CoV-2 Test	Lab	13-Aug-20		X				X	X	X		
Yale School of Public Health, Department of Epidemiology of Microbial Diseases	SalivaDirect	Lab	15-Aug-20	X									X
DxTerity Diagnostics, Inc.	DxTerity SARS-CoV-2 RT-PCR Test	Lab	21-Aug-20	X	X	X							X
Guardant Health, Inc.	Guardant-19	Lab	21-Aug-20	X					X	X	X		
Texas Department of State Health Services, Laboratory Services Section	Texas Department of State Health Services (DSHS) SARS-CoV-2 Assay	Lab	21-Aug-20	X	X				X	X	X	X	
Fluidigm Corporation	Advanta Dx SARS-CoV-2 RT-PCR Assay	Kit	25-Aug-20	X									X
Cuur Diagnostics	Cuur Diagnostics SARS-CoV-2 Molecular Assay	Lab	26-Aug-20	X	X	X			X	X	X		
Patients Choice Laboratories, LLC	PCL SARS-CoV-2 Real-Time RT-PCR Assay	Lab	28-Aug-20	X	X	X			X	X			
BayCare Laboratories, LLC	BayCare SARS-CoV-2 RT PCR Assay	Lab	31-Aug-20		X		X		X				
MiraDx	MiraDx SARS-CoV-2 RT-PCR assay	Lab	31-Aug-20	X					X	X	X		
Mammoth Biosciences, Inc.	SARS-CoV-2 DETECTR Reagent Kit	Kit	31-Aug-20	X					X	X	X		
T2 Biosystems, Inc.	T2SARS-CoV-2 Panel	Kit	31-Aug-20	Not specified					X	X	X	X	
Detectachem Inc.	MobileDetect Bio BCC19 (MD-Bio BCC19) Test Kit	Kit	1-Sep-20	X			X		X	X	X		

Format = "Kit" is an in vitro diagnostic available for purchase by qualified labs, "Lab" indicates testing must be performed within that laboratory; Date = Date of EUA approval; LRT specimens include bronchoalveolar lavage fluid, tracheal aspirates, bronchial washings, and sputum. "URT other" specimens include nasal washes and mid-turbinate swabs. Data current as of Sept 17, 2020.

Assays

At time of writing, there are 41 serological assays available approved under the EUA in the United States [43]. For most clinical purposes, the assays that are available have comparable performance for the detection of antibodies to SARS-CoV-2. Despite this, the assay design varies considerably between manufacturers. Supply chain issues that have at times plagued molecular testing have not affected serological assays for SARS-CoV-2. This may be due to the lessened demand for serology relative to diagnostic testing.

Assay format

Commercially available assays may take several formats; 1) chemiluminescent immunoassays that are generally high throughput and run on large, commercially available analyzers available in most clinical laboratories, 2) enzyme-linked immunosorbent assays (ELISA) which are typically performed on 96-well plates in a manual or semi-automated fashion and 3) lateral flow assays which resemble pregnancy tests. There are potential benefits and detriments to each assay design. For example, chemiluminescent immunoassays generally have high reproducibility and larger throughput than other methods. However, they do not generate titers, which are the gold standard when assessing protection and response to infection [44]. ELISAs can be easily titered, but their lower throughput, especially when generating a titer is a major limitation in most clinical laboratories. Finally, lateral flow-based assays may potentially be useful, particularly in seroprevalence studies or in low-resource areas. However, these assays frequently lack sufficient clinical sensitivity and specificity for detection of antibodies to SARS-CoV-2 [36]. Importantly, only one serological assay has been cleared for use at POC at the time of writing [43]. Hospitals may use EUA lateral flow serological methods that are considered moderately complex at the bedside under a CLIA license. However, sites such as physicians' offices that perform testing under a CLIA Certificate of Waiver are only authorized to perform lateral flow testing on devices authorized for POC testing [45]. Finally, neutralization assays, which detect the presence of neutralizing antibodies to SARS-CoV-2 have also been developed [46]. However, neutralization assays require either Biosafety level 3 facilities using relatively low-throughput methods or the creation of a pseudovirus-based assay. No neutralization assays have been approved by the FDA for clinical use at this time.

Classes of immunoglobulins detected

Available assays detect anti-SARS-CoV-2 IgG, IgM, IgA, or total antibody. When assessing previous exposure to SARS-CoV-2, there is currently no known benefits to any one specific assay design [47]. Frequently after exposure to a virus, seroconversion with IgM occurs several days to weeks before IgG leading some to speculate that IgM would provide enhanced sensitivity for detection of acute SARS-CoV-2 infections. However, this has not been proven to be true in patients with COVID-19. In a study of 26 patients tested longitudinally, 9/26 patients seroconverted IgG and IgM simultaneously and 10/26 seroconverted IgG prior to IgM [48]. Similarly, Zhao et al. observed near simultaneous median time to positivity of 12 days for IgM and 14 days for IgG using assays not yet available in the US [49]. Furthermore, total antibody assays which identify several classes of antibodies have similar clinical sensitivities for the detection of anti-SARS-CoV-2 antibodies relative to assays which only detect IgG. Tang et al. demonstrated comparable performance between the Roche anti-SARS-CoV-2 total immunoglobulin assay, the Abbott anti-SARS-CoV-2 IgG assay, and the EUROIMMUN anti-SARS-CoV-2 IgG assay at $>$ 14 days post symptom onset and at $<$ 14 days post symptom onset [50,51]. Similarly, Harb et al. observed comparable sensitivities in convalescent plasma between assays that target total anti-SARS-CoV-2 immunoglobulin and those that target IgG. These studies imply that neither total immunoglobulin nor the detection of IgM improve the sensitivity of serological assays for early detection of patients with COVID-19 infections. It is important to note that the IDSA recommends against the use of assays for SARS-CoV-2 IgA. This is primarily due to the low observed sensitivity and specificity [52]. The IDSA also recommends against the use of combination IgG or IgM tests, which are assessed separately, but wherein only one of the two are positive [53]. This is primarily due to concerns of enhanced cross reactivity of IgM and lower specificity relative to IgG, reducing overall specificity. Manufacturers' claims for sensitivity and specificity can be found on the FDA website [43], but

independent studies have demonstrated lower sensitivities in hospitalized patients likely due to differences in patient populations.

Viral antigen targets

Commercially available serological assays for SARS-CoV-2 also detect antibodies to different viral antigens. The vast majority of assays detect antibodies directed against the nucleocapsid protein, the spike protein, or the receptor-binding domain (RBD) region of the spike protein. While the nucleocapsid protein tends to be more highly immunogenic than the spike protein [47], studies have yet to demonstrate conclusive differences between clinical assays that target antibodies to different SARS-CoV-2 proteins [50,54]. Although the nucleocapsid protein is more highly conserved across coronaviruses than the spike protein, clinical assays have demonstrated similar specificities. As a result, the IDSA and the CDC make no recommendation at this point regarding the antigenic target used [29,53].

Utility

Despite the availability of serological assays for SARS-CoV-2 exceeding several months, the clinical utility is still relatively narrow. The proposed utilities include 1) diagnosis of acute infection, 2) seroprevalence studies and 3) determining protection after previous exposure.

Diagnosis of acute infection

Early in the course of the pandemic, serological assays were proposed to be an important supplement to diagnostic testing [55]. This was due to supply chain issues cited previously for diagnostic molecular methods and numerous appeals that serology may play a role for diagnosing acute infection [49,56]. However, serological assays have poor sensitivity for detection of antibodies to SARS-CoV-2 early after symptom onset. Theel et al. have previously demonstrated sensitivities of <50% and <11% at 8–14, and <8 days post symptom onset respectively using the Abbott, Epitope, EUROIMMUN, and Ortho-Clinical SARS-CoV-2 serological assays [54]. Similarly, Tang et al. demonstrated sensitivities of ~42% with Roche, ~31% with Abbott, and 33% with the EUROIMMUN SARS-CoV-2 assays in patients with <14 days post-symptom onset. As previously noted, detection of IgM antibodies to SARS-CoV-2 does not seem to provide enhanced clinical sensitivity. At our institution, patients with SARS-CoV-2 typically present to the ED within 3–4 days from symptom onset, well before seroconversion would be anticipated. Therefore, it is not advised to use SARS-CoV-2 serological assays for assessing the presence of acute infection [29,53]. However, some symptomatic patients may present a week or later after symptom onset and are persistently negative by PCR. The CDC recommends that serological testing is used as an adjunct to diagnostic molecular testing if a patient presents more than 9 days from symptom onset [29]. However, the IDSA recommends against the use of serology until 14 days after symptom onset, citing a pooled sensitivity of 68% in patients from 7 to 14 days [53]. Pediatric patients with a multisystem inflammatory syndrome, a disease presenting with Kawasaki-like features including fever and shock, may benefit from the use of serological assays to confirm diagnosis. In a study of 95 confirmed cases in NY, 47% were diagnosed by positive serology in the absence of positive molecular testing [57]. Thus, while serological assays should not be used for acute diagnosis, they have limited clinical utility in a subset of patients.

Unsurprisingly, patients that are immunocompromised often fail to seroconvert or have longer time to seroconversion relative to immunocompetent patients after SARS-CoV-2 infection. A study of 21 patients with chronic lymphoblastic leukemia revealed only 67% seroconversion to IgG at a minimum of 28 days following the symptom onset [58]. Similarly, studies have demonstrated lower seroconversion rates among SARS-CoV-2-infected cancer patients relative to health care workers [59]. However, little is available in the peer-reviewed literature among patients with rheumatic diseases. A case study of two patients with MS treated with ocrelizumab both failed to mount an immune response at 6 and 7 weeks after symptom onset [60]. Importantly, the presence of autoimmune diseases and potentially cross reacting antibodies does not seem to effect the specificity of serological assays for SARS-CoV-2 [50,54,61]. Further studies are needed to confirm the time to seropositivity in this potentially at-risk population and the sensitivity of commercially available assays in immunosuppressed patients. However, given the likelihood of a lower test sensitivity, negative results from a

serological assay should be interpreted with caution in patients receiving immune modulating therapies.

Seroprevalence studies

SARS-CoV-2 serological assays have been used to assess the seroprevalence within a population. These studies have important ramifications for public health policy including understanding the burden of disease particularly due to limited early testing by molecular methods and to quantitate the mortality rate among infected individuals. Several studies have demonstrated that the seroprevalence of SARS-CoV-2 is 2−10 times greater than the number of patients who have been reported positive by molecular methods [62,63]. For seroprevalence studies, it is crucial for the test to have high specificity and positive predictive value (PPV), particularly for an emerging virus with a relatively low prevalence [34]. For example, in a population of 1,000,000 people with a prevalence of 1%, a test with a sensitivity and specificity of 99% respectively would yield PPV of ~50%. Thus, one in two positive results would be a false positive. As a result, the CDC advocates for the use of an assay with a specificity >99.5% or an orthogonal approach by which all positive results are tested again using a secondary method [29]. While seroprevalence studies have limited clinical application for individual patients, many patients are interested in their serostatus. Likely as a result of marketing from manufacturers and underlying public interest, the vast majority of serological tests at our institution are performed in outpatient settings. However, in settings with low pretest probability and low prevalence (i.e., outpatient physician office in a patient with limited previous symptoms), a method with high specificity is required, similarly to seroprevalence studies. Therefore, it is important for clinicians to understand the limitations of the assay (i.e., known specificity) and the approximate prevalence in the area when serological assays are used for assessing previous exposure in asymptomatic patients.

Protection after previous exposure

If SARS-CoV-2 serological testing is performed, it is important to provide clear information to the patient regarding the utility of the result, particularly when positive. One of the most important discussions regarding COVID-19 serology is if a positive result equates to protection from future SARS-CoV-2 infections. This has been phrased as an "immunity passport," which would permit previously infected patients with the presence of antibodies to travel, return to work, and generally resume life as normal with the presumption of immunity. However, the degree of protection and duration of immunity offered by previous exposure to SARS-CoV-2 is still relatively unknown. As a result, the CDC, IDSA, and WHO all recommend against the use of serological assays for determining immune status [29,53,64]. Nonetheless, there is mounting evidence that infection with SARS-CoV-2 confers some degree of protection. Rhesus macaques infected with SARS-CoV-2 were protected from reinfection 35 days following the initial exposure [65]. Interestingly, three fisherman who were previously infected with SARS-CoV-2 had no evidence of viral infection and experienced no symptoms after subsequent exposure from an outbreak on a fishing vessel [66]. Specimens from the exposed fisherman that were drawn prior to re-exposure all demonstrated the presence of neutralizing antibodies. This implies that exposure and the generation of neutralizing antibody titers are sufficient for protection from reinfection. However, there are several limitations with presuming that positive serological results for anti-SARS-CoV-2 assays equates to long-term immunity. For example, Tang et al. previously demonstrated that hospitalized patients that died or had worse outcomes had higher neutralizing antibody titers than hospitalized patients with improved outcomes, implying that neutralizing titers may not associate with improved outcomes or protection [67]. Furthermore, a study of convalescent plasma from 149 individuals revealed neutralizing titers <1:50 in 33% of patients [68]. In contrast, the early recommendation from the FDA was a minimum neutralizing titer of 1:160 with an ideal of 1:320 for convalescent plasma donors [69]. Moreover, neutralizing titers may not be the mechanism of protection, with several studies demonstrating the importance of T cells [70,71]. Another problem with inferring protection from future SARS-CoV-2 infection with serological assays is that they serve as an imperfect proxy for neutralization. One study found a negative percent agreement (NPA) of 55% or less using a neutralizing cutoff of 1:128 as positive compared to positive serological results from three high-

throughput, commonly used clinical assays [67]. Similarly, other authors demonstrated a NPA of 32% with neutralizing assays relative to a commercially available ELISA [72]. While there is a modest correlation between the signal generated on commercially available serological assays and neutralizing assays, most assays are qualitative. While quantitative SARS-CoV-2 serological assays are emerging, correlations are required with these assays relative to neutralizing antibody titers. Another remaining question is the durability of the immune response to SARS-CoV-2. Several studies have demonstrated that circulating antibody concentrations decrease considerably within the first 90 days from symptom onset, particularly in patients with mild and asymptomatic infections [73–75]. Importantly, longitudinal studies of seasonal coronaviruses have found that patients are frequently reinfected with the same coronavirus, often within 12 months of the previous infection. Longitudinal studies assessing the durability of antibodies to SARS-CoV-2 are needed.

Future role of serology

As the pandemic resumes, serological assays are being developed and used for various purposes. Serological assays have been implemented to identify convalescent plasma donors with presumably high titers of antibodies. Early results from the expanded access program for convalescent plasma have demonstrated that patients who receive convalescent plasma units with higher levels of antibody as identified by the Ortho-Clinical SARS-CoV-2 IgG assay have better outcomes relative to those with lower levels of antibodies [76]. Serological assays may also provide value in identifying vaccinated individuals and the sufficiency of the humoral response once a vaccine is available. Since vaccinations target portions of SARS-CoV-2 spike protein, it will be crucial for providers to know the antigenic target of the assay when used for this purpose. Finally, algorithm-based approaches using serological assays that target multiple antigens and immunoglobulin classes may be useful in future. An algorithmic approach is used for hepatitis B virus serology, which allows for discrimination between recent infection, previous infection, and immunity [77]. However, no such algorithm has been proposed or endorsed by professional societies for SARS-CoV-2.

Summary

SARS-CoV-2 is an RNA betacoronavirus that is responsible for the current COVID-19 pandemic. Laboratory evaluation for diagnosis and surveillance has been rapidly developed to assist treatment public health efforts to prevent its spread. However, with the heightened awareness that laboratory testing is crucial for this function, hundreds of platforms have been made available in a relatively short period of time. Many of these platforms have distinct advantages to offer, including turnaround time, sensitivity, types of specimens accepted, ease of use, availability, and cost. False-negative results can occur due to a number of factors. These can be analytical and also preanalytical, before the specimen even arrives in the laboratory. Molecular-based testing is the only diagnostic assay format authorized by the FDA, but it is still only qualitative, and can vary greatly in assay sensitivity. Serological testing is more useful for monitoring seroprevalence, while its role in assessing protective immunity is still under investigation. In addition to laboratory-based testing options, clinicians will continue to be presented with numerous point-of-care assays to choose from, and these will suffer from similar challenges faced by the central laboratory. While we have made tremendous progress in making effective testing available to those most in need of it, we continue to face unanswered questions related to the future role of molecular and serological testing. For example, we do not fully understand how viral load is associated with clinical outcomes and if a quantitative test would therefore be useful. Still unknown is how the durability of the serological response differs in natural infection compared to vaccination. Importantly, the humoral immune response to SARS-CoV-2 in patients with rheumatologic diseases and on immune modulating therapies requires further evaluation. Laboratory testing for SARS-CoV-2 will continue to be confronted by numerous logistical challenges that influence options for patient testing.

Practice points

- The sensitivity of FDA EUA molecular diagnostic assays varies widely and depending upon the platforms, it may not accurately capture individuals with low viral loads.
- False-negative test results can derive from issues within the laboratory or outside the clinical testing environment, such as inadequate sample quality.
- The clinical utility of serological assays for SARS-CoV-2 is currently relatively limited but may aid in diagnosis in rare cases.

Research agenda

- Prospective studies describing the association between quantitative molecular diagnostic results and outcomes are needed to interpret the significance of the viral load.
- Association between protection from future SARS-CoV-2 infections and serological assays positive for the presence of antibodies to SARS-CoV-2 requires further studies, particularly those that assess the durability of the serological response.
- The development of algorithm-based approaches to serological testing to assess for acute infection, chronic infection, and vaccination may provide enhanced value to serological testing to SARS-CoV-2.
- Further studies are required assessing the humoral immune response to SARS-CoV-2 in patients with rheumatologic diseases and on immune modulating therapies.

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Declaration of competing interest

No conflicts of interest are declared by the authors.

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