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REVIEW

Molecular Diagnostic Tools for the Detection of SARS-CoV-2

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ABSTRACT

The pandemic causing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has globally infected more than 50 million people and ~1.2 million have succumbed to this deadly pathogen. With the vaccine trials still in clinical phases, mitigation of Coronavirus Disease 2019 (COVID-19) relies primarily on robust virus detection methods and subsequent quarantine measures. Hence, the importance of rapid, affordable and reproducible virus testing will serve the need to identify and treat infected subjects in a timely manner. Based on the type of diagnostic assay, the primary targets are viral genome (RNA) and encoded proteins. Currently, COVID-19 detection is performed using various molecular platforms as well as serodiagnostics that exhibit approximately 71% sensitivity. These methods encounter several limitations including sensitivity, specificity, availability of skilled expertise and instrument access. Saliva-based COVID-19 diagnostics are emerging as a superior alternative to nasal swabs because of the ease of sample collection, no interaction during sampling, and high viral titers during early stages of infection. In addition, SARS-CoV-2 is detected in the environment as aerosols associated with suspended particulate matter. Designing virus detection strategies in diverse samples will allow timely monitoring of virus spread in humans and its persistence in the environment. With the passage of time, advanced technologies are overcoming limitations associated with detection. Enhanced sensitivity and specificity of next-generation diagnostics are key features enabling improved prognostic care. In this comprehensive review, we analyze currently adopted advanced technologies and their concurrent use in the development of diagnostics for SARS-CoV-2 detection.

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Biosensor; converging technology; COVID-19; point-of-care diagnostics; virus

Introduction

Coronaviruses [CoV] were acknowledged in the late sixties with a distinctive corona or crown of sugary proteins emanating from the virion surface. CoV belong to the order Nidovirales and *Coronaviridae* family. These viruses contain a large (~30 kb), positive sense, single stranded (ss) RNA genome and may encode up to 25–30 different proteins [1]. Annotated as highly recombinogenic, CoV may possess subregions in the genome with independent origins. The first Alpha HCoV-229E discovered in 1960s, infected immunocompromised individuals with mild symptoms [2].

Beta coronaviruses are categorized into three lineages viz., A, B and C. Lineage A contains two members, HCoV-OC-43 [1967] and HCoV-HKU [2005] [3,4]. Symptoms presented by the members of lineage A include acute rhinitis, lower respiratory tract infection, and gastrointestinal infections. These community-acquired viruses are estimated to account for 5–15% of all common cold infections. Lineage B includes SARS-CoV, which was discovered in 2002/2003 and exhibits severe symptoms such as fever, cough, chills, myalgias, and leads to Acute Respiratory Distress Syndrome [ARDS] [5]. Lineage C is represented by Middle East Respiratory Syndrome [MERS] discovered in 2012, and clinical manifestations range from cough, severe pneumonia, and ARDS to fatality [6]. Another genetically similar representative of lineage B, capable of infecting and causing mortality in humans, SARS-CoV-2, has become the source of the pandemic in 2020. This novel candidate is symptomatically similar to the common cold, as well as MERS and SARS-CoV. SARS-CoV-2 shares 96% sequence identity with a bat coronavirus and 79.5% sequence identity with SARS-CoV. Phylogenetic analysis suggests that SARS-CoV-2 is likely a zoonotic transfer from bats to humans via an intermediate host. Individuals with co-infections of pneumonia, HIV, or

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influenza present an additional challenge for SAR-CoV-2 testing [7]. Furthermore, analysis of the spike glycoprotein gene bears marked similarity with the gp120 and gap proteins of HIV-1 at four distinct sites [8]. Although no sequence homology has been found between influenza virus and SARS-CoV-2, clinical similarities may be misleading in preliminary diagnosis of COVID-19. Hence, there is an urgent requirement for the development of point-of-care diagnostics (POCD) that can generate diagnostic specificity.

COVID-19 emerged as an infectious disease with its epicenter in Wuhan, China. The first publicly reported case was identified in the Hubei district of China in November 2019. Based on its growing pandemic nature, the World Health Organization [WHO] declared the 2019–20 SARS-CoV-2 outbreak a pandemic and public health emergency of international concern. As of November 2020, more than 50 million cases and 1.2 million deaths have been reported worldwide. Currently, SARS-CoV-2 exhibits maximal virulent efficacy amongst all the members of *Coronaviridae*. Intriguingly, significantly higher fatality rates have been confirmed in geriatric patients with comorbidities like hypertension, diabetes, cardiovascular disease, chronic respiratory disease and various types of cancer [9]. Confirmed positive subjects with any aforementioned comorbidity yielded poorer clinical outcomes compared to those with no known comorbidity.

POCD enable rapid confirmations, thus creating the potential to accelerate medical care. Additionally, it has been observed that SARS-CoV-2 can persist in aerosols for up to 3 h. The virus particles generated by coughing and sneezing remain suspended in the air or may attach to suspended particulate matter, thus enabling transmission via air. This phenomenon may have contributed to community spread of the disease. Therefore, consistent monitoring will aid detection of viral persistence via aerosols and enable control of viral spread and active infections. In this review, we provide insights into SARS-CoV-2 targets and the future of efficient diagnostics for detection of this pandemic virus.

Diagnostic targets in SARS-CoV-2

Coronaviruses are enveloped, spherical viruses ranging from 150 to 160 nm in size [4]. The viral genome is comprised of ssRNA (~29–32 kilobases long) with varying GC content (32% to 43%) [9,10]. SARS-CoV-2 distinctively differs from other coronaviruses (CoV) by encoding a supplementary glycoprotein that has acetyl esterase and hemagglutination [HE] properties [11].

As of October 2020, more than 5100 genomes have been sequenced for SARS-CoV-2 showing 1–6 single nucleotide mutations at different loci [using Wuhan-Hu-1/2019 as reference genome: Acc no *NC_045512.2*]. The viral genome contains a unique N-terminal fragment within the spike protein and genes for the major structural proteins characteristic of all coronaviruses exist in the 5′–3′ order as spike [S], membrane [M], envelope [E], and nucleocapsid [N] proteins [2]. A typical CoV exists with at least six open reading frames [ORFs] in its genome. All the structural and accessory proteins are translated from the subgenomic (sg) RNAs of CoV and four major structural proteins S, M, E and N are prearranged by ORFs 10, 11 on one-third of the genome near the 3′-terminus [10]. These mature proteins are primarily accountable for several vital functions in genome safeguarding and virus replication (Figure 1).

Some of the viral strains have demonstrated both synonymous and nonsynonymous amino acid substitutions in the Nucleocapsid (N), Membrane (M), and Spike (S) proteins as well as proteins encoded by ORF1a, ORF1b, ORF3a, ORF8 and ORF14 [10]. The strains can now be distinguished into 10 distinct clades with each accumulating mutations over time (Figure 2).

Virus-encoded glycoprotein S, a major antigen present on the viral coat, is expressed as a class I fusion protein composed of 1,300 amino acids. Specific amino acid residues (473–486) in the receptor-binding domain of S enable interaction of this glycoprotein to its ligand, angiotensin-converting enzyme (ACE)-2 receptor. The binding affinity of *coronaviridae* S protein with its ligand determines the rate of viral replication, transmissibility and disease severity. Binding of S-ACE-2 depicts equilibrium dissociation constants of 1.2 and 5.0 mM for SARS-CoV-2 and SARS viral species indicating a strong affinity with ACE-2 receptors [12].

Another major protein in the virus is N, composed of three distinct yet conserved domains: namely the N-terminal domain (NTD/domain 1) and C-terminal domain (CTD/domain 3), joined by RNA-binding domain/domain 2. This protein has six immunodominant epitopes and is actively involved in viral replication and genome packaging. N protein can be detected in gargle specimens and nasopharyngeal swabs. Most diagnostic methods generally target the N and S proteins of SARS-CoV-2 for detection.

The next target that enables the virus to manifest its infection is an RNA-dependent RNA polymerase [RdRp]. This protein is part of a multiprotein

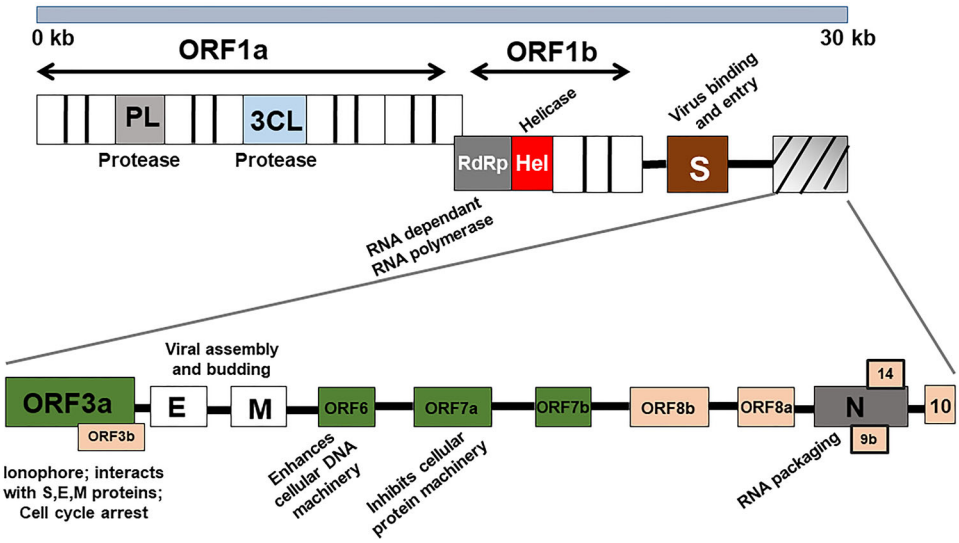


Figure 1. Schematic representation of SAR-CoV-2 genome organization. The virus encodes around 28 different ORFs that perform functions related to viral genome replication, virion formation, virus entry, and immune evasion. Key ORFs with their reported functions are highlighted on the genome.

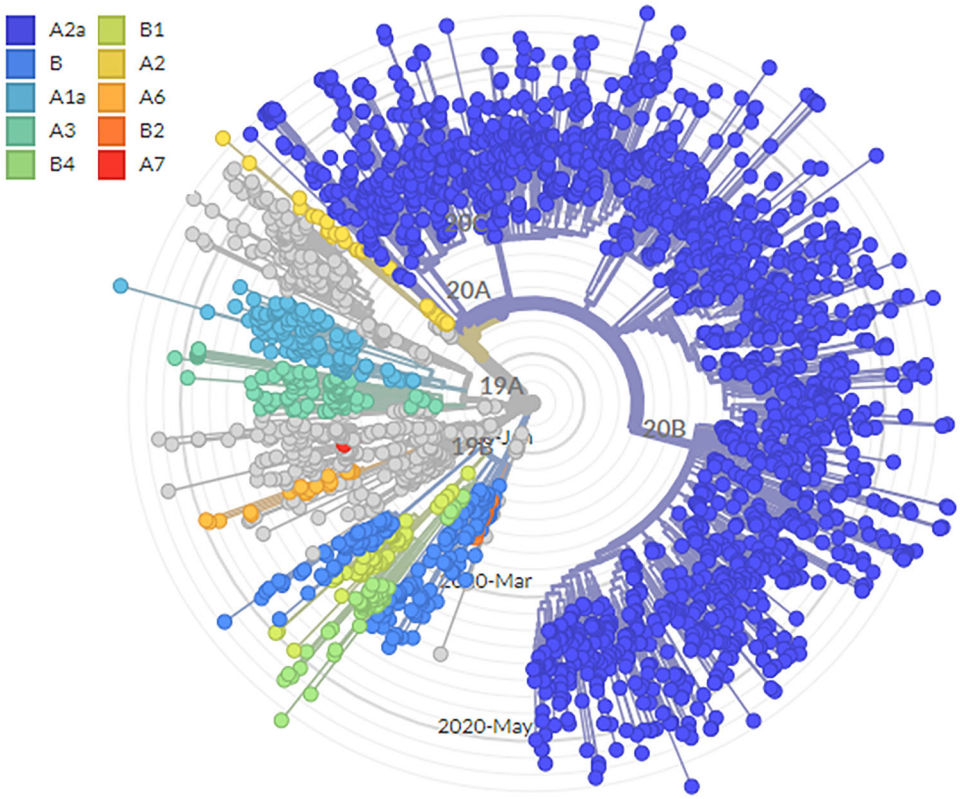


Figure 2. Evolutionary relationship of SARS-CoV-2 quasispecies from the COVID-19 pandemic [Image generated by nextstrain.org]. Phylogeny indicates an initial emergence in Wuhan, China, in November–December 2019 followed by sustained human-to-human transmission (data pertains to sampled infections). The figure indicates a spatiotemporal depiction of the mutations occurring at the genomic level resulting in the emergence of genetic variability. Temporal resolution indicates mutation rate of 8×10^{-4} subs per site per year. Each color designates a different clade in which organisms have diverged from the ancestral Wuhan strain. Distance from origin indicates the period of divergence for different strains in each clade.

complex containing 500–600 amino acid residues embedded in the membrane. RdRp complex consists of an RNA polymerase, nsp12, whose processivity is augmented by nonstructural protein 7 [nsp7] and nsp8. The RdRp catalytic module generates full-length [–] RNA copies of the genome and uses it as a

Table 1. Samplers used for testing of aerosolized SARS-CoV-2 virus.

Type of sampler	Volume captured	Time	References
SASS 2300 Wetted Wall Cyclone Sampler	300 L/min	30 min	[20]
Cyclone bioaerosol sampler	3.5 L/min	4 h	[21]
Sterilized gelatin filters with pore size 3 μm placed in styrene filter cassette	5 L/min	1 h	[19]

template for the synthesis of full-length [+] RNA genomes [13]. High error rates, typically about 1/34,000, in viral RdRp's copying ensure enormous sequence variation in the RNA virus population, allowing rapid virus evolution under selective pressures imposed by the host immune response and/or drug treatments. Strand switching during RdRp copying is also a mechanism for RNA recombination, allowing RNA viruses to repair deleterious mutations, rearrange genes, and acquire new genes from other viruses or their host(s) further facilitating virus to evolve and mutate rapidly [14].

Indispensable enzymes 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro) enable the activation of the viral replication complex. In B lineage CoV, PLpro additionally enables viral infection by stripping ubiquitin and ISG15 from host proteins to aid coronaviruses in their evasion of the host innate immune responses [15]. PLpro aids inhibition of IFN-B induced by mitochondrial antiviral signaling protein, and concomitantly reduced NF-kB activity induced by TNF-α. Virus-encoded 3CLpro is a major target for inhibitor studies, as it is crucial for viral replication through its proteolytic processing of polyprotein 1ab [pp1ab] at 11 different cleavage sites. Polyproteins pp1a and pp1ab contain the nsp 1–11 and 1–16, respectively [16]. This compact virus thus presents multiple targets at both the genomic and protein levels for efficient detection.

Overall, a combination of viral genome and highly expressed, indispensable viral proteins (S, N, RdRp, M, 3CLpro, etc.) can be targeted for efficient detection of the virus using various molecular platforms.

Environmental prevalence and detection

Viral persistence and transmission as aerosol-dispersed variants has given rise to a new arena of virology known as aerovirology. SARS-CoV-2 is frequently detected in droplet nuclei as well as on the surface of objects and suspended particles, thus augmenting viral transmission [17]. The half-life of the SARS-CoV-2 on aerosols is about 0.64–2.64 h [18–21]. Coughing humans emit microdroplets with a size ranging from 0.6 to 15 μm. These have a tendency to remain airborne for longer periods with the settling velocity varying from $3{\cdot}1 \times 10^{-3}\,\mathrm{ms}^{-1}$ for 10 μm particles to $3{\cdot}5 \times 10^{-5}\,\mathrm{ms}^{-1}$ for 1 μm particles. Droplets larger than 10 μm are not able to cross the upper airways. On the contrary, smaller particles travel toward the lungs and are trapped in trachea–bronchial and alveolar pockets. Typical RNA concentration quantified in the throat and sputum samples of COVID-19 patients is $\sim 10^4 - 10^{11}$ copies/mL. SARS-CoV-2 infected subjects, without wearing facemasks, can spread virus to other individuals within 6 feet of range or in enclosed spaces.

Two methodologies, namely wet air and dry air, are used for sampling; they are followed by either reverse-transcriptase quantitative PCR (RT-qPCR) or nested PCR for confirmatory testing [22]. Few positives were obtained, thus highlighting the necessity of sampling for airborne viruses capable of causing respiratory distress. A similar study was first performed for SARS-CoV-2 in the COVID-19 isolation ward of a hospital in Milan, Italy and air samples collected from the contaminated area were all found to be positive for SARS-CoV-2 [23]. Efficient detection of SARS-CoV-2 in the air is feasible if proper sampling is attained, which is possible if the sampler and the sampling environmental conditions are considered simultaneously. Different types of samplers like liquid impingers, solid impactors, filters, and electrostatic precipitators are also employed to detect presence of viruses in the air [23]. Samplers use polytetrafluoroethylene (PTFE) membrane filters, HEPA filters polycarbonate (PC), and gelatin filters for trapping the suspended viral particles. Erstwhile collection devices known as Biosampler, Cyclone sampler, and the MD-8 air (Table 1) scan sampling device have been used to capture different members of *coronaviridae* from the air and are currently employed to capture SARS-CoV-2 [22].

In addition to environmental transmission, wastewater is now being considered as a conduit to estimate SARS-CoV-2 community spread. SARS-CoV-2 RNA, present in patient feces, increases the viral load in sludge. Monitoring viral copies in wastewater can provide a glimpse of viral spread in the community. A specific RT-qPCR-based study targeting N1 and N2 genes conducted in New Haven metropolitan area detected SARS-CoV-2 RNA in concentrations ranging from 1.7×10^3 -4.6×10^5 virus RNA copies /ml of primary sludge. Wastewater surveillance through

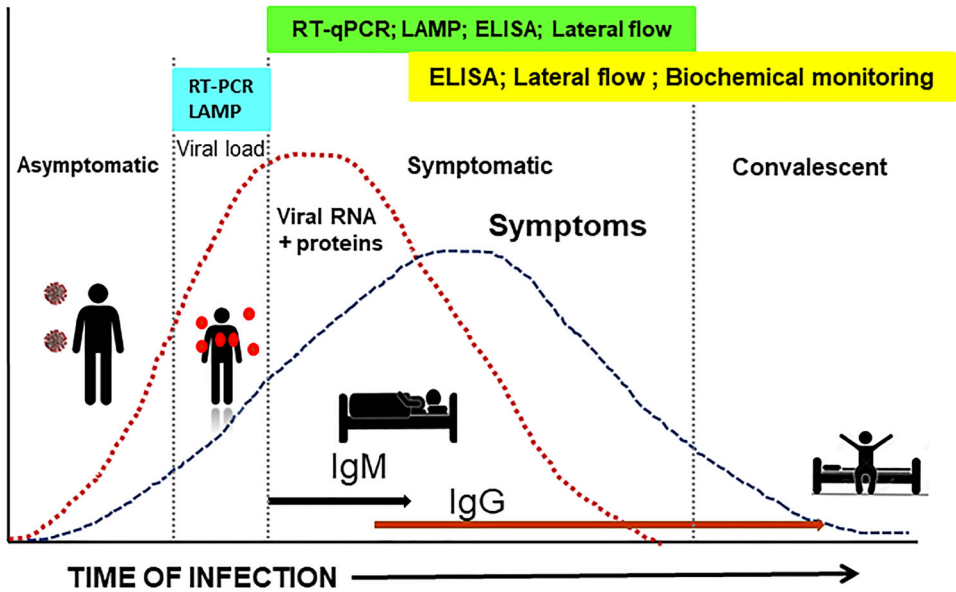


Figure 3. Schematic of progression of COVID infection and in vitro diagnostics used at various stages of severity. Viral load and evolution of symptoms are represented as red and blue dotted lines. *X*-axis represents the time of infection; as the incubation period differs in individuals, it is not labeled.

charting out a correlation between viral shedding and dynamics could generate an epidemic model and track community infections in the area. Transmission dynamics may enable timely healthcare interventions and ease the pressure on the health-care system [24,25].

Diagnostics

Approximately 25% of the SARS-CoV-2 infected individuals are asymptomatic, yet are "virus shedders" [26]. Hospital-based diagnostics like CT are accessed only by symptomatic candidates upon recommendation of a healthcare professional. Apart from being expensive, CT exposes the patients to high doses of radiation which may in some cases elicit additional allergic reactions. For successful control of the pandemic, it is important to screen asymptomatic/pre-symptomatic subjects in their incubation period with the same precautionary steps required for symptomatic patients [27]. This gap may be bridged by bringing POCDs bedside, allowing individuals to utilize it as a prognostic tool without any specialized training. The advent of new technologies has facilitated integration of a multidisciplinary, system-wide approach; thus, we rely on converging technologies (optics, electronics, wireless technology, nanotechnology, etc.) to design practical indicators of quality and safety in situ. Some of the diagnostics in current use are depicted in Figure 3.

PCR-based diagnostics

RT-qPCR is the gold standard and currently the most predominant method used to detect SARS-CoV-2 infection. Sensitivity and specificity of RT-qPCR relies heavily on the expertise of the individual performing the assay and the RNA quality of the samples collected from the lower and upper respiratory tract. Moreover, single negative samples do not rule out SARS-CoV-2 infection. The majority of infected individuals remain asymptomatic during the viral incubation period. Once the virus emerges from the incubation period, sample collection and processing are crucial as the sensitivity of the RT-qPCR will depend on the viral load [28]. Limit of detection (LoD), defined as the lowest target amount any analytical test can detect, is a mechanism to assess the efficacy of a kit.

RT-qPCR sampling relies on Oropharyngeal swabs (OPS), saliva, or nasopharyngeal swabs (NPS) for confirmatory coronavirus detection. Results have confirmed better accuracy with NPS samples compared to OPS or saliva. Various countries have developed their own SARS-CoV-2 specific RT-qPCR kits. For instance, Germany targeted RdRp, E, and N transcripts; China designed primers targeting ORF1ab and N transcripts; Hong Kong targeted nsp14 and N transcripts; Japan targeted multiple ORFs, and Thailand developed kits targeting N transcripts [29]. Variants of PCR, like RT-insulated isothermal PCR [RT-iPCR] and an one-step RT-PCR assay based on a highly sensitive *Taq*man Probe remain the current mechanisms of diagnosis [27,30].

Another methodology commercialized in 2011, Droplet Digital PCR (ddPCR), is similar to qPCR with some minor twists. First, the PCR reaction is partitioned into thousands of individual reaction vessels prior to amplification and the data is acquired at reaction end-point. ddPCR has a distinct advantage of minimizing the effect of contamination while maintaining efficacy in quantifying cDNA in samples [31]. ddPCR has confirmed higher sensitivity and specificity than qPCR for false-negative samples with low viral load with an LOD equivalent to 0.00187 ng. Although ddPCR is more costly and time consuming than qPCR, higher sensitivity makes it a better choice for diagnosing COVID-19 [32].

Loop-mediated Isothermal Amplification (LAMP) is a single tube reaction generally used for the detection of DNA and RNA sequences. This technique uses a combination of 4–6 primers against different loci of target DNA sequences with the reaction temperature varying between 60 and 65 °C. The RT-LAMP assay has a significant edge given that SARS-CoV-2 is an RNA virus with length of about 30 kB and combined reverse transcription (RT) and LAMP together significantly diminishes detection time. An amplified product was detected via phenol red-based photometry whereby detection limit of the colorimetric RT-LAMP assay could detect a threshold cycle (CT) $\approx$ 30. Another group led by Xiaojing Li demonstrated that the RT-LAMP assay in clinical samples had a high specificity (99.5%), sensitivity (91.4%) and a positive predictive value (97.7%). An advanced methodology, swab-to-RT-LAMP assay, uses NPS and OPS and exhibited a high sensitivity for samples with a CT $<$ 25. New England Biolabs (NEB) has developed a Quick Colorimetric LAMP Kit, which may be used to detect novel coronavirus RNA. The kit acts as a simple alternative to RT-qPCR and enables visual discovery of amplification of SARS-CoV-2 nucleic acid in only 30 min [33,34].

Serology-based diagnostics

The presence of antibody indicates that an immune response has developed in the person exposed to the pathogen. Serology testing is recommended only for observational purposes and not for diagnostics. However, the information on the intensity of adaptive immune response activation can provide insights into an individual's ability to restrict virus and may be critical in understanding virus pathobiology. Serologic assays are important in cases where RNA may be difficult to isolate or is no longer present, and for epidemiological studies [27]. Sensitivity may be categorized as analytical and diagnostic. Analytical sensitivity is defined as the ability of a test to analyze antigen or antibody concentration in a target biological sample, whereas diagnostic sensitivity is a test's sensitivity with true positive rate against incidence of disease. Accuracy, an indispensable term in diagnostic development, is defined as the number of true positives and true negatives in targeted sampling [27,35,36].

Coronavirus presents four structural surface proteins: S, M, N and E proteins. Most of the kits detect IgM/IgG specific against N, S and RBD proteins present in human serum and plasma [26]. Serological tests may be broadly categorized into four types; rapid diagnostic tests, chemiluminescent immunoassays, ELISA and neutralization assays. The sensitivity of serotests is generally lower than molecular methods and is predominantly used for retrospective diagnosis. Earlier, ELISA-based detection of antibodies against MERS-CoV was developed, which is based on N and S proteins of MERS-CoV [37]. Currently, serology-based kits for SARS-CoV-2 have been generated by various diagnostic firms and have been granted emergency-use approval (EUA) by the U.S. Food and Drug Administration (FDA) in the wake of the pandemic. Epitope Diagnostics Inc, USA [38] has developed both IgG and IgM-based ELISA tests: it utilizes Horse Radish Peroxidase [HRP]-conjugated immune complexes of COVID-19 antigen and antibody [IgG/IgM] and is performed on a spectrophotometric microplate reader (450 nm). Similarly, Eagle Biosciences has developed IgM-based COVID-19 testing kits, while EUROIMMUN has developed IgA and IgG-based serological tests. However, these kits are still awaiting FDA approval. One such diagnostic developed by Promega, USA, is Lumit™ Dx Immunoassay. It consists of two fragments of SARS-CoV-2 Spike protein receptor binding domain are labeled with two complementary subunits of the NanoBiT® Luciferase viz., Lumit™ Dx CoV-2-SmBiT and Lumit™ Dx CoV-2-LgBiT. NanoBiT® Luciferase is an engineered enzyme that is ~100-folds brighter than conventional luciferase thereby increasing assay sensitivity. Upon incubation with a SARS-CoV-2 positive sample, antibodies tend to bring SmBiT and LgBiT together thus forming an active luciferase. Luminesce emitted is amplified by an additional additive and detected in a microplate reader. The test has shown 93% specificity and 100% sensitivity [39]. Countries including Germany, Japan, Spain, China, Singapore, and USA have generated EUA and FDA approved serological diagnostics.

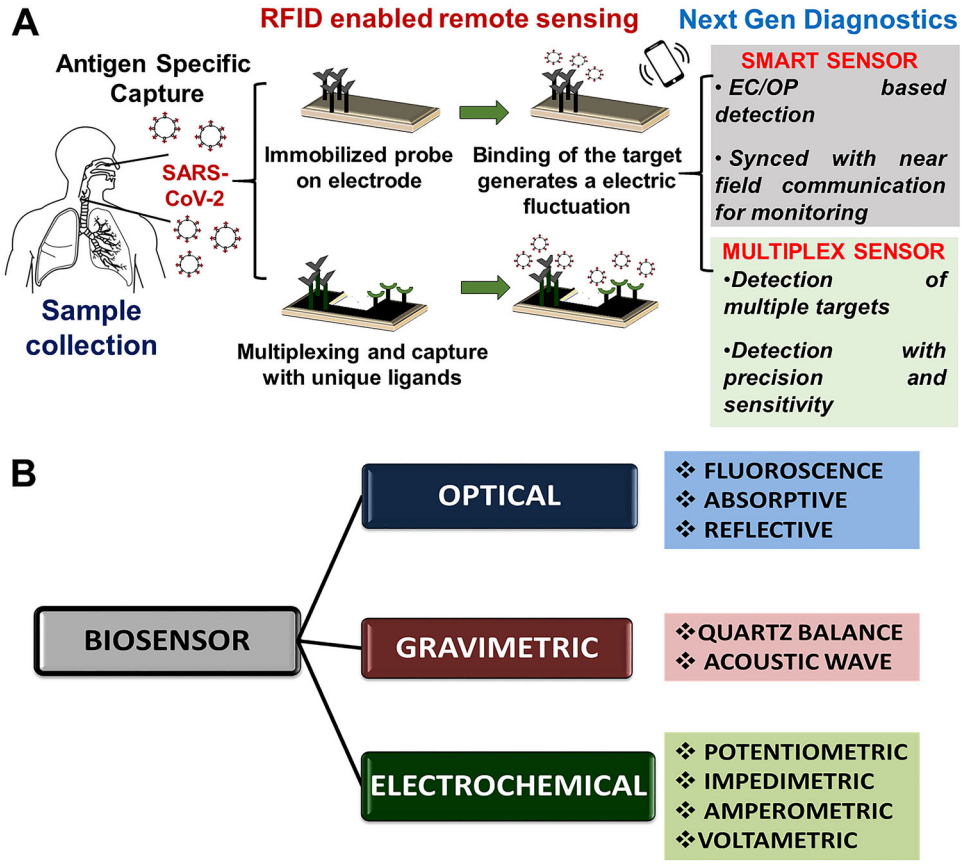


Figure 4. (A) Schematic depicting biosensor-based detection and data integration using real time monitoring platforms. (B) Different classes of biosensors with available transducers that may be integrated to develop point-of-care diagnostics by leveraging available converging technologies.

Biosensors-based diagnostics

Biosensors are a diagnostic platform that detect any molecule by generating signals directly proportional to analyte(s) concentration. Figure 4A depicts a schematic for the detection of SARS-CoV-2 using various types of biosensors. Their sensitivity and selectivity depend on the affinity of the analyte to the probe and its dissociation constant [Kd]. Complementarity of the target molecule with the ligand forms the basis of interaction. Polynucleotide-based aptamers, molecular imprints, lectins, proteins, and antibodies are frequently utilized as recognition elements [RE] for binding. Any fluctuation in the physicochemical parameters or the interaction may transduce as a signal [40]. Transducer-based biosensors may be classified broadly as optical, gravimetric, and electrochemical sensors (Figure 4B).

Optical biosensors utilize light to monitor interaction (binding or a reaction) between a probe and an analyte by determining changes in light absorption post-interaction. Label-free optical sensor may percept the change in refractive index and thus confirm binding of the analyte–probe molecule. Alternatively, optical sensors can utilize fluorescent tags that have the capability to produce or quench a signal upon interaction between analyte and probe. Gravimetric biosensors use the basic principle of a response to detect change in mass due to binding between probe and analyte. Acoustic-based gravimetric sensors perceive the alteration in resonating frequency corresponding to the binding of the analyte and probe and thus the increase in mass of the bimolecular complex. Electrochemical sensors detect the change in electron flow between interacting molecules and represent it as resultant change in electric current [41]. An interaction between two biological macromolecules often results in the flux of electrons. Leveraging electron level changes may reveal molecular interactions, which thereafter may be assessed quantitatively or semi-quantitatively. Various interacting partners like DNA–DNA, DNA–RNA, DNA–protein, and protein–-ligand have been envisaged for designing electrochemical biosensors as *in vitro* diagnostics.

Many sensor platforms have been designed and validated for different members of the *Coronaviridae* (Table 2). Global initiatives are underway to develop

Table 2. Biosensors designed for coronaviruses.

Type of sensor	Ligand	Capture	Advantage	Disadvantage	Refs.
Imaging ellipsometry sensor	Antibodies	SARS-CoV-2	Analyze minimum two and maximum 16 parameters at each wavelength. No reference sample is required.	Optical Model is required to characterize each thickness layer of a sample.	[47]
Microfluidic protein nanopattern sensor	Envelope protein	SARS-CoV-2 Ab	Offer high throughput processing, sensitive to real time detection with accuracy.	Sensitive to adjacent environment. Redox elements are required to increase diagnostic sensitivity.	[48]
Optofluidic nanoplasmonic sensor	Group-specific antibodies	SARS-CoV Virus	Rapid and sensitive virus detection technique. Sample preparation not required.	Background shifting, extensive optimization required for the surface chemistry.	[47]
DNA-tile based self-assembling nano sensor	DNA	SARS viral DNA	Real time detection with Nanoelectronics/nanophotonic devices and protein/ ligand nanoarrays.	Requires improvement and optimization of tile assembly modeling	[49]
Luciferase-based biosensors	RLKGG peptide	MERS CLpro and PLpro	Shorter turnaround time with sensitivity of the LucN-LBD-LucC biosensor.	Detection sensitivity is not highly accurate	[50]
Colorimetric DNA sensor	Pyrrolidinyl Peptide Nucleic Acid	MERS DNA	Highly selective for the complementary oligonucleotides over single-base-mismatch. Simple, rapid, sensitive and selective DNA detection	Efficacy limited Doubtful Reproducibility Expensive	[50]
LSPR	DNA probe-AuNP	MERS DNA	Sensitive, high biocompatibility, SPR sensors with cost effective	Poor efficacy due to the high optical spectral ring	[51]

sensors enabling rapid detection of SARS-CoV-2. Most of the designed sensors are using an electrochemical-based detection platform. An electrochemical field-effect transistor (FET)-based biosensor has been designed to detect the SARS-CoV-2 spike protein *in vitro*. Three different samples (antigen protein, cultured virus, and nasopharyngeal swab) were analyzed and performance of the sensor was quantified. The device could detect the S protein at concentrations equivalent to 1 fg/mL in 60 s, whereas nasopharyngeal swab [42] could detect 100 fg/mL. A two-dimensional (2D) heterostructure made up of tellurene, indium tin oxide, and functionalized molybdenum disulfide (MoS2) layers for sensitive detection of SARS-CoV-2 and S protein was designed. The composite used ACE-II enzyme for capturing the targets and demonstrated an excellent linear detection range for S glycoprotein (301.67 nM) and SARS-CoV-2 (67.8762 nM) [43]. Another group at UC Santa Cruz is using 'nanopipette' technology to design a POCD for COVID-19 testing. A four-channel nanosensor chip can detect four different proteins simultaneously and formation of target–probe complexes tends to disrupt the flow of electric current passed through the nanopipette-like opening. Sensitivity is approximately 1 fm/ml and is due to receive EUA approval soon. As compared to other serological and molecular tests, this sensor would be 4–10 times cheaper and 20–50 times faster in detection [44].

A gold pillar and graphene-based electrochemical sensor was designed and was synced with a smartphone-based user interface. This aerosol jet (AJ) nanoparticle 3D printing device contains 3D agglomerated gold nanoparticles embedded in a microfluid and can detect antibodies specific to SARS-CoV-2 S1 protein RBD. In addition to its picomolar detection, an added advantage is probe regeneration by lowering the pH, which can be achieved in less than one minute. This assures platform reusability. This sensor has been validated at the preclinical level and has been undergoing trials for approval [45]. A gold nano-island–glass composite was immobilized with DNA receptors complementary to SARS-CoV-2 specific RNA. Detection was based on two different concepts to detect the virus safely and reliably: an optical and a thermal. Localized surface plasmon resonance (LSPR) detected the change in the local refractive index when the SARS-Cov-2 specific RNA bound the DNA probe. The RNA–DNA hybrid resulted in localized heat production when excited with laser of a certain wavelength and this could be used to distinguish between viral nucleotide sequences [46].

CoNVaT is a diagnostic sensor for COVID-19 aiming to detect virus from the first day of infection. This nanophotonic biosensor is a bipartite device: one detects virus proteins and the other viral RNA (genetic material). The sensitivity of the designed biochip is attributed to the principle of photonic interferometry whereby light undergoes small but perceptible changes upon intersecting with an object, thus confirming the presence of virus in samples [52].

CRISPR-based in vitro diagnostics

Clustered-Regularly Interspaced Short Palindromic Repeats (CRISPR) and its associated protein, Cas,

system comprise a genome editing tool capable of targeting multiple genomic loci simultaneously. This in vivo editing tool has been extensively used to study gene function and perform genomic manipulation, thus revolutionizing biological research. The CRISPR/Cas system is currently being employed for the detection of various malignancies [53] and scientists have also developed diagnostics for the detection of coronaviridae. Specific High-sensitivity Enzymatic Reporter Unlocking (SHERLOCK) utilizes a CRISPR molecule to detect the SARS-CoV-2 genetic signature in nasal swabs, nasopharyngeal swabs, oropharyngeal swabs or bronchoalveolar lavage (BAL) specimens. Detection of signature sequences results in activation of the CRISPR enzyme, resulting in release of a detectable fluorescent glow [54]. A modified version of SHERLOCK, All-in-one dual CRISPR/Cas12a (AIOD-CRISPR) assay is a one-pot, one-temperature reaction that uses two Cas12a-crRNA constructs mediating continuous cleavage and increase in amount of fluorescent ssDNA-FQ reporter [55]. The signal can be detected in the presence of UV light or LED blue light. Another CRISPR-based IVD, DETECTR assay, is dependent on a Cas substitute, Cas12a, and targets the E and the N2 gene of SARS-CoV-2. Viral RNA is initially amplified using fluorescent tags and exposed to a Cas12a-sgRNA complex. Binding of the complex to the target results in ssDNA cleavage activity, resulting in an increase in fluorescently labeled ssDNA substrates. Lateral flow then amplifies the detection signal generated by interaction between biotin–streptavidin tags. Permutations and combinations of CRISPR, variants of Cas and different targets in the viral DNA have been used to generate two more IVDs, namely CREST and FELUDA, which are yet to be validated. Each of the IVDs can detect minute quantities of RNA (ranging up to femtomolar) within 40–60 min, thus facilitating rapid and sensitive identification [55,56]. CRISPR-based COVID assay sensitivity depends on the efficiency of sample collection and extraction methods and hence similar limitations will be persistent as are associated with molecular techniques. Approximately 0.21% of mutations in the SARS-CoV-2 may also add to discrepancies in detection via CRISPR-Cas techniques.

Pros and cons of different SARS-CoV-2 diagnostics methods

With the passage of time, assorted variants of IVDs (Table 3) have been designed and developed. Permutations and combinations of different probes and targets (nucleic acids or serology-based) have been used to increase specificity and sensitivity of detection (Figure 5A). RT-PCR is considered the gold standard of SARS-CoV-2 detection, although false negatives have been observed when viral copies are too low to be amplified. The viral copies differ due to the efficacy and site of sample collection in addition to the number of days post-infection the sample was collected. The tests may be conducted only in labs approved by Clinical Laboratory Improvement Amendments of 1988 (CLIA), hence the restrictions in the number of tests which may be performed successfully. Immunological tests rely on the presence of antibodies in serum. IgM is generally detected 7 days post-infection and peaks after 28 days, whereas IgG is detected in a period of 10–49 days after infection. Hence, a test done before 7–10 days may return as negative [57]. Each of these tests have their own shortcomings (Figure 5B) and the research community is still striving to attain the epitome of COVID-19 diagnostics.

Conclusion

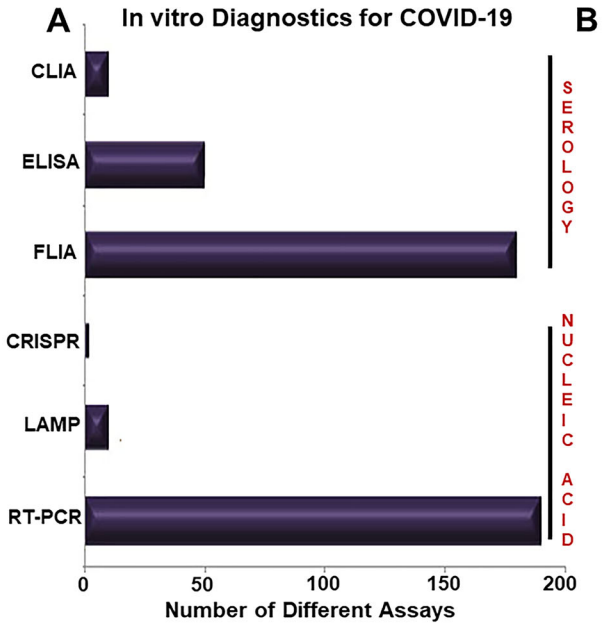
Numerous diagnostics have been developed or are under development to cope with the burgeoning requirement of society during the pandemic. However, with a remarkable increase in the recent global COVID-19 positivity rate, rapid and efficient testing is critical to overcome this pandemic. Screening each individual frequently (weekly, biweekly, or monthly) for the presence of viral genome or antigens could be extremely significant in containing the virus spread, yet remains a challenge. In addition, this would reveal hitherto unknown virus pathobiology that can be a resource in developing and testing treatment modalities and facilitate monitoring the recovery of infected individuals.

According to the WHO, CDC, FDA, there are currently no significant medications that have proven effective for the treatment or prevention of SARS-CoV-2, in particular for public usage. However, from the earlier case studies of the management of SERS-CoV and MERS-CoV, many drugs have been repurposed for the treatment of COVID-19. Across the globe, health practitioners are testing the permutations and combinations of antivirals, corticosteroids and derivatives of chloroquinone [60–63]. In addition, researchers are trying different strategies to develop the vaccine against COVID-19 to find the ultimate cure for this dreaded virus. GISAID (Global Initiative on Sharing All Influenza Data), a nonprofit

Table 3. COVID testing kits under current use [57–59].

Type of test	Kits available	Company	Approval	Targets
Serology tests				
Total Ab-ECLIA	Elecsys Anti SARS-CoV-2 S	Roche Diagnostics	USFDA EUA	SARS-CoV-2 Ag
Lateral flow IgM/G	Innovita 2019-nCoV Ab Test (Colloidal Gold	Innovita (Tangshan) Biological Technology Co., Ltd.	USFDA EUA	SARS-CoV-2 Ag
Total antibody, CLIA	Dimension EXL SARS-CoV-2 Total antibody assay (CV2T)	Siemens Healthcare Diagnostics Inc.	USFDA EUA	SARS-CoV-2 Ag
IgM-CLIA	Access SARS-CoV-2 IgM	Beckman Coulter, Inc	USFDA EUA	SARS-CoV-2 Ag
Total Ab-ELISA	OmniPATH COVID-19 Total Antibody ELISA Test	Thermo Fisher Scientific	USFDA EUA	SARS-CoV-2 Ag
IgM-CLIA	Diazyme DZ-Lite SARS-CoV-2 IgM CLIA Kit	Diazyme Laboratories, Inc.	USFDA EUA	SARS-CoV-2 Ag
Antigen-based diagnostics				
Sandwich IA	Sampinute COVID-19 Antigen MIA	Celltrion USA, Inc	US FDA EUA	N
Lateral flow IA	CareStart COVID-19 Antigen test	Access Bio, Inc.	US FDA EUA	N
Lateral flow IFA	Sofia 2 Flu + SARS Antigen FIA	Quidel Corp.	US FDA EUA	N
Lateral flow IA	BinaxNOW COVID-19 Ag Card	Abbott Diagnostics Scarborough	US FDA EUA	N
Microfluidic IFA	LumiraDx SARS-CoV-2 Ag Test	LumiraDx UK Ltd.	US FDA EUA	N
Chromatographic digital IA	BD Veritor System for Rapid Detection of SARS-CoV-2	Becton, Dickinson and Company	US FDA EUA	N
One-step immunoenzymatic ("sandwich") assay	Access IL-6	Beckman Coulter, Inc	US FDA EUA	IL6
IA	Elecsys IL-6	Roche Diagnostics	US FDA EUA	IL6
Nucleic acid amplification tests				
RT-PCR	OPTISARS-CoV-2 RT PCR Test	OPTI Medical Systems, Inc	US FDA EUA	N1, N2
RT-PCR	Abbott Real Time SARS-CoV-2	Abbott Diagnostics, Inc	WHO EUL; US FDA, EUA; CE-IVD	RdRp, N
RT-PCR	COVID-19 genesig Real-Time PCR assay	Primerdesign Ltd.	WHO EUL; US FDA EUA; CE-IVD	RdRp
RT-PCR	Lyra SARS-CoV-2 Assay	Quidel Corp.	US FDA EUA	Pp1ab
RT-PCR	Cobas SARS-CoV-2 Test	Roche Molecular Systems, Inc	WHO EUL; US FDA EUA	ORF1ab, E
RT-PCR	PCR Fluorescence Probing-2019nCoV-Nucleic Acid Diagnostic Kit	Sansure Biotech, Inc	US FDA EUA; CE-IVD; NMPA (China)	ORF1ab, N
RT-PCR	STANDARD M nCoV Real-Time Detection kit	SD Biosensor Inc	US FDA EUA; CE-IVD; MFDS EUA (Korea)	ORF1ab, E
RT-PCR	*Taq*Path™ COVID-19 Combo Kit	Life Technologies	US FDA EUA; CE-IVD	ORF1ab, N, S
RT-LAMP	AQ-TOP™ COVID-19 Rapid Detection Kit	Seasun Biomaterials, Inc	USFDA EUA	ORF1ab
Isothermal RT-PCR	ID NOW COVID-19	Abbott Diagnostics Scarborough,	USFDA EUA	RdRp
Isothermal RT-PCR	Cue COVID-19 Test	Cue Health Inc	USFDA EUA	N
Isothermal RT-PCR	Pro-AmpRT SARS-CoV-2 Test	Pro-Lab Diagnostics	USFDA EUA	ORF1ab
Isothermal RT-PCR	iAMP COVID-19 Detection Kit	Atila BioSystems, Inc.	USFDA EUA	ORF1ab, N
RT-LAMP	Loopamp New Coronavirus 2019 (SARS-CoV-2) Detection Reagent Kit	LAMPEiken Chemical	USFDA EUA	Replicase 1B region
RT-LAMP + CRISPR	SARS-CoV-2 DETECTR Reagent Kit	Mammoth Biosciences	USFDA EUA	RP and N Gene
CRISPR	SOLARIS Multiplex SARS-COV-2 ASSAY	Solaris Diagnostic	USFDA EUA	N1 and N2

*IA = immunoassay; IFA = immunofluorescence assay; RT = reverse transcription; LAMP = loop mediated isothermal amplification.



B

	ADVANTAGES	DISADVANTAGES
CLIA	• Higher Sensitivity And Specificity •Broad Linearity • Automated	• Need Supporting • Chemiluminescence Instruments
ELISA	• Simple Operation • Low Cost, • High-throughput	• Time-consuming • Vulnerable To Contamination
LFIA	• Simple Operation • Low Cost • Rapid	• Low Sensitivity During Early • SARS-COV-2 Infection
CRISPR	• High Specificity • High Precision • High Efficiency • Simple And Fast Operation	• Experienced Manpower • Confirmation May Require Multiple Swab Collections From An Individual
RT-LAMP	• On-site Detection • Simple Operation • Cost Effective	• Low Throughput
RTPCR	• High Sensitivity And Specificity	• Sophisticated Equipment • Experienced Operators

Figure 5. Approved diagnostic tests for COVID-19 testing commercially available and the associated advantages and disadvantages with each type of test [57,59].

organization hosted as a public private partnership between Germany, Singapore and the United States of America, has taken initiative to generate a data sharing platform so that genome sequences from all over the world become globally accessible [64]. As of December 2020, almost 52 vaccines are at different phases of clinical evaluations, whereas more than 160 candidates are at various stages of development and testing. These vaccine platforms consist of inactivated virus particles, protein subunits, nonreplicating viral vectors, viral RNA and DNA, and attenuated virus [65]. Out of these, six vaccine candidates have received authorizations and approval in various countries. The frontrunner of the approved vaccine, BNT162b2, is an mRNA-based vaccine developed by BioNTech and Pfizer. It generates an immune response against the mutated form of the full spike protein of the virus encoded by the vaccine. This vaccine has obtained approvals in UK, UK, Bahrain, Canada, Mexico, US. Three candidates from China, namely, CoronaVac (Sinovac), BBIBP-CorV (Beijing Institute and Sinopharm), and another vaccine developed by Sinopharm and two from Russia namely, Sputnik V and EpiVacCorona are the other vaccines which have obtained EUA in their countries. Additionally, Moderna, Cansino Biologics, Astrazeneca, Bharat Biotech, Johnson & Johnson and Novavax are conducting phase 3 trials for their promising vaccine candidates [66].

Cure may be successfully implemented when there is an optimized platform for timely detection of the disease. Biosensor-based SARS-CoV-2 detection can overcome the current challenges in detection (sensitivity and accuracy) and prolonged virus tracking, and can facilitate multiplexed pathogen detection in asymptomatic individuals. Hence, the role of biosensor and CRISPR-based IVDs as POCD is indispensable in mitigating SARS-CoV-2. Biosensors and CRISPR-based IVDs may enable selective and sensitive detection without the requirement of a highly trained workforce and may thus provide timely intervention in curbing the COVID-19 pandemic.

Equally significant is the global initiative to share public health information, diagnostic platforms, biobanks and datasets to advance the knowledge in containing the virus, especially during the pandemic. European infrastructure for translational medicine (EATRICS) is providing a collaborative niche of "clinical, biological and technological expertise" by engaging European Clinical Research Infrastructure network (ECRIN) and the European research infrastructure for biobanking (BBMRI) for an initiative known as COVID-19 fast response service. EATRICS is enabling the researchers and physicians to collaborate and advance the field of biomedical innovations aimed at virus detection that will improve disease management and treatment. Numerous IVDs have been developed, yet very few have been approved by FDA postvalidation. Most of the diagnostics currently being used have been granted emergency utilization authorization (EUA) whereas many have been granted research use only (RUO). EUA endorsements are

being generated on the basis of performance data sheets being submitted by laboratories seeking approval, which includes data about the type of sample being considered, test principles and performance evaluation based on analytical sensitivity, and cross-reactivity amongst other issues [67,68].

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

MD and ARN conceived the concept; DDS prepared figures and/or tables; MD, DDS, and ARN contributed to writing of the manuscript. All authors reviewed and approved the final draft.

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