Infection
https://doi.org/10.1007/s15010-022-01819-6

REVIEW

Coronavirus: a comparative analysis of detection technologies in the wake of emerging variants

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Received: 2 February 2022 / Accepted: 30 March 2022

Abstract

An outbreak of the coronavirus disease caused by a novel pathogen created havoc and continues to affect the entire world. As the pandemic progressed, the scientific community was faced by the limitations of existing diagnostic methods. In this review, we have compared the existing diagnostic techniques such as reverse transcription polymerase chain reaction (RT-PCR), antigen and antibody detection, computed tomography scan, etc. and techniques in the research phase like microarray, artificial intelligence, and detection using novel materials; on the prospect of sample preparation, detection procedure (qualitative/quantitative), detection time, screening efficiency, cost-effectiveness, and ability to detect different variants. A detailed comparison of different techniques showed that RT-PCR is still the most widely used and accepted coronavirus detection method despite certain limitations (single gene targeting- in context to mutations). New methods with similar efficiency that could overcome the limitations of RT-PCR may increase the speed, simplicity, and affordability of diagnosis. In addition to existing devices, we have also discussed diagnostic devices in the research phase showing high potential for clinical use. Our approach would be of enormous benefit in selecting a diagnostic device under a given scenario, which would ultimately help in controlling the current pandemic caused by the coronavirus, which is still far from over with new variants emerging.

Keywords SARS-CoV-2 · Infectious disease · Variants · COVID-19 · Point-of-care · Diagnostic devices

Introduction

Severe Acute Respiratory Syndrome (SARS) caused by a beta coronavirus abbreviated as SARS-CoV-2 and coronavirus disease 2019 (COVID-19) [1, 2] rapidly spread worldwide. World Health Organisation (WHO) declared COVID-19 as a pandemic in March 2020. The cases are still rising, and by March 20, 2022, 472 million cases and 6.1 million deaths were recorded [3]. With the emergence of more infectious strains, the future regarding control of disease and accurate diagnosis is unknown. Complete vaccination of the public will take months, and vaccines are not effective against each variant. Under these scenarios, the diagnosis of COVID-19 holds immense importance [4].

Symptoms expressed by COVID-19 patients are not consistent over different people and vary with mutations in the virus; hence, they cannot be used as accurate criteria for screening. Thus, it is important to have quick, accessible and accurate onsite point-of-care diagnostic devices for timely diagnosis, so that infected patients can be isolated and treated to curb infection and mortality rates [5].

This study aims to collate different diagnostic techniques, their corresponding devices and classify them based on screening efficiency, detection limit and effect of mutations on detection. We have further discussed some of the methods in the research phase that are not clinically used to detect COVID-19.

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Published online: 26 April 2022

Existing diagnostic methods and devices

Reverse transcription polymerase chain reaction (RT-PCR)

SARS-CoV-2 ribonucleic acid (RNA) is reverse transcribed into complementary deoxyribonucleic acid (cDNA), and specific gene fragments are amplified using target-specific primers [6] by polymerase chain reaction (PCR). Amplified cDNAs are quantified using probes [7] emitting readable fluorescent signals. Amplification is important to detect a small amount of virus among large genetic information. The detection method of RT-PCR is summarized in Fig. 1. RT-PCR requires specific instruments and is generally carried out in the laboratory [8, 9].

Two years after the pandemic, RT-PCR is still considered the gold standard for detecting COVID-19 [10]. The most common samples for RT-PCR are throat and nasopharyngeal swabs [11]. RT-PCR has sensitivity of 98% and specificity of 95–100%, and can detect within 3 h. Sensitivity of RT-PCR increases considerably after day-6 of illness [12]. More than 150 commercialized RT-PCR COVID-19 diagnostic kits are developed worldwide [13] and some major ones are discussed in Table 1.

United States (US) developed a one-step RT-PCR that uses gene-specific and region-specific probes. Many other countries have also developed gene-specific COVID-19 testing kits. Some of them are Altona Diagnostics (Germany) [20], CerTest biotech (Spain) [25], and Seegene (Korea) [23]. Corman et al. [26] generated a novel in vitro transcribed RNA standard that accurately matches the sequence of SARS-CoV-2, thereby increasing the sensitivity. Tang et al. [27] used stool specimens to detect COVID-19 by RT-PCR with 59% accuracy. COROSURE (IIT Delhi, India) RT-PCR Kit [16] detected COVID-19 using a probe-free method, considerably reducing the cost to USD 9 without compromising the accuracy. COVIRAP [17], RT-PCR kit developed by IIT Kharagpur, India uses a paper strip to detect DNA of the SARS-CoV-2 which can be interpreted by a mobile application. It shows 94% sensitivity and 96% specificity. GeneXpert system which was earlier used to detect other diseases like tuberculosis and HIV has now been approved by the US Food and Drug Administration for emergency use in COVID-19 detection, which can detect with a sensitivity of 100% and specificity of 80% [24].

Some limitations of RT-PCR are long-term nucleic acid extraction, requirement of trained staff, errors during sample preparation, and high cost for large volumes. A few RT-PCR kits can also fail to differentiate between influenza virus and SARS-CoV-2.

Computed tomography scan (CT-scan)

For COVD-19-infected patients, a CT-scan of lungs shows infiltrates, ground-glass opacities, and sub-segmental consolidations (Fig. 2). CT-scan has higher sensitivity (86–98%) and fewer false-negative results than RT-PCR [28]. CT-scan imaging supported decision-making, provided immediate isolation and appropriate patient treatment [29]. CT-scan combined with other diagnostic techniques provides better diagnosis during the early stages of infection [30]. CT-scan is limited as the lungs are not always the infected organ. COVID-19 can cause multi-organ dysfunctions [31]. Parameters associated with COVID-19 infection observed in CT-scan are also not specific to COVID. The imaging time for a CT-scan is longer, is expensive, and requires a radiologist to analyze the results. In addition to this, CT-scan uses ionizing

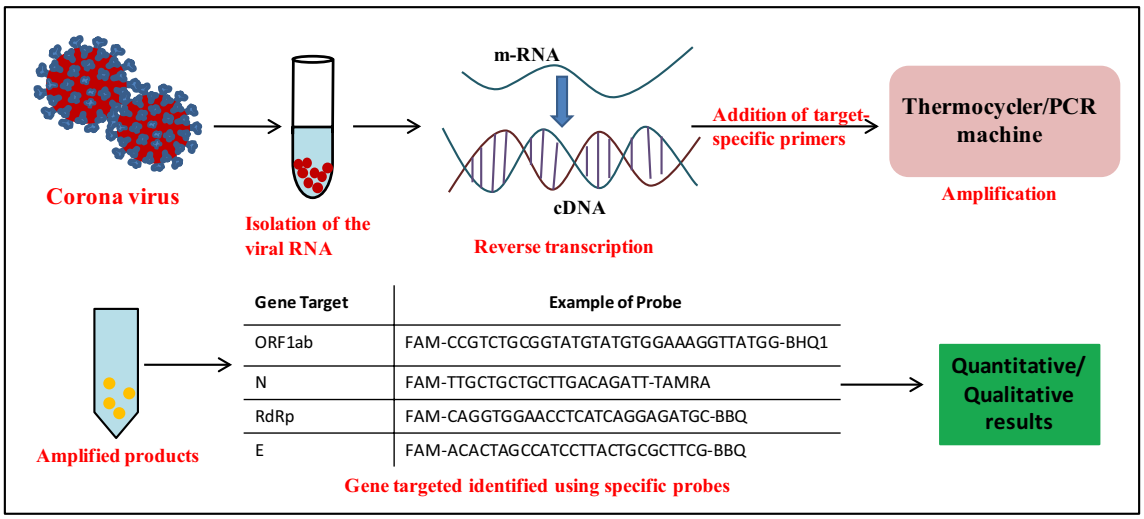

Fig. 1 An overview of the procedure of RT-PCR with sequences of some probes [7]

Table 1 Comparison of kits based on RT-PCR for COVID-19 detection

S. No.	Name of the test/kit	Approved by	Principle	Reagent/biomarkers/genes targeted	Sample type	Time of detection	Novelty/other specific information	Accuracy/specificity/sensitivity/LOD	Cost
(1)	1copy™ COVID-19 qPCR Multi Kit 1 drop [14]	Korea MFDS EUA; US FDA EUA; Health Canada; Saudi FDA; Sri Lanka NMRA; CE-IVD	Qualitative detection Real-time detection	RNA dependent RNA Polymerase genes (RdRp) and E gene	Nasopharyngeal and Oropharyngeal swab	1 h 50 min	Very low limit of detection	Sensitivity—100% to 10^3 copies LOD—4copies/reaction	USD 6.2
(2)	TRUPCR SARS-CoV-2 RT-qPCR Kit 3B Black Bio Biotech India Limited [15]	India CDSCO; US FDA EUA	Qualitative detection Assay contains target-specific probes, labeled by fluorescent reporter and quencher dyes for detection	E, RdRP and N gene in two tube format	Nasopharyngeal Swabs, Oropharyngeal samples	60–90 min	Two tube format and single step detection from RNA	No- cross-reactivity, false-negative results	~ USD 10
(3)	COROSURE (IIT Delhi) [16]	ICMR India	Quantitative detection Probe- free method- using a comparative sequence analysis—identifying short stretches of RNA in the covid-19 genome	S1 and S2 gene	Serum, nasopharyngeal swabs	50–60 min	Probe-free method reduces its cost (no compromise with accuracy)	Sensitivity and Specificity 100%	USD 5.5
(4)	COVIRAP (IIT Kharagpur) [17]	ICMR India	Qualitative Isothermal Nucleic acid detection. Extraction of RNA by alternate to specialized equipment. Replacement of paper cartridge for each test	Not specified	Saliva samples	45 min	Portable automated pre-programmable temperature control unit detection unit on a simple strip of paper, No manual intervention	Sensitivity 94% and Specificity 98%	USD 6.64

Table 1 (continued)

S. No.	Name of the test/kit	Approved by	Principle	Reagent/biomarkers/genes targeted	Sample type	Time of detection	Novelty/other specific information	Accuracy/specificity/sensitivity/LOD	Cost
(5)	Abbott Real Time SARS-CoV-2 assay Abbott Laboratories [8]	Singapore; Brazil; US	Qualitative method The device gives higher throughput and narrows the manual steps with sturdy results. Reduced cold-chain logistics as well	N gene and RdRp gene	Nasal, nasopharyngeal and Oropharyngeal swabs	470 samples in 24 h-	Max ratio analysis of data helps to remove operator subjectivity	Dual target assay for RdRp and N gene	USD 10
(6)	SARS-CoV-2 Real Time PCR Detection Kit Zena Max-Advance Molecular Diagnostics AMD [18]	United Kingdom	Qualitative detection The FAM and ROX fluorescence signal becomes detectable above quantification cycle	RdRp and N gene	Nasopharyngeal swabs, oropharyngeal swabs, saliva	1 h	No cross-reactivity. Can be incorporated into ready-to-use PCR. Ensure maximum sensitivity	Sensitivity—minimum one copy/reaction Specificity-100% for Human SARS-CoV-2 Virus RNA	Not available
(7)	COVID-19 qPCR-I Kit AIT biotech [19]	Singapore; South Africa SAHPRA; CE-IVD	Quantitative real-time PCR detection Detection of two COVID-19 specific regions from non-structural polypeptide in a single reaction	Non-structural protein1 (np1) (ORF 1a) and non-structural protein 2 (np2) (ORF1a)	Nasal aspirate, Nasopharyngeal swabs Bronchoalveolar lavage, Tracheal aspirate, Sputum or Serum	1.5 h	Size per kit—50 samples	Sensitivity—2.2 copies/µl or 11 copies/reaction	USD 127 for 50 samples
(8)	RealStarSARS-CoV-2 RT-PCR Kit ALTONA diagnostics [20]	CE-IVD	Qualitative in vitro real-time PCR technology for differentiation of lineage B-βCoV and SARS-CoV-2 specific RNA	E gene and S gene	Serum and saliva samples	1–2 h	For research use only (RUO). Reagent system for Internal and positive control	Not Provided	USD 1592 for 300 samples

Table 1 (continued)

S. No.	Name of the test/kit	Approved by	Principle	Reagent/biomarkers/genes targeted	Sample type	Time of detection	Novelty/other specific information	Accuracy/specificity/sensitivity/LOD	Cost
(9)	Kylt SARS-CoV-2 Kylt Diagnostics [9]	CE-IVD	Quantitative RT-PCR Detection of SARS-CoV-2 Viral RNA. FAM and HEX for fluorescent signal	Not specified	Naso and Oropharyngeal Swabs, Sputum, Bronchoalveolar Lavage	20–30 min	Detect different variants, with high specificity, sensitivity and accuracy. Size per kit-100	LOD—< 10 copies per microliter of RNA	Not available
(10)	Bio-Rad SARS-Cov-2 ddPCR BIO-RAD [21]	US FDA EUA; Philippines FDA; RUO	End-point RT-PCR qualitative detection of nucleic acids from SARs-CoV-2	N gene, Human RNase P gene	Nasopharyngeal swabs Aspirate and nasal aspirate	1 h 30 min	It is a 2019-n CoV CDC digital droplet PCR Triplex Probe Assay	Sensitivity 0.260 cp/µl to 0.351 cp/µl (cp = copies)	Not available
(11)	COVID-19 PCR kit My Biosource [22]	WHO; US CDC	Quantitative RT-PCR detection The synthesis of cDNA occurs in a single qPCR	ORF1ab and N gene	Human respiratory specimens	less than 2 h	Includes a Positive Control. Ready to use for research pertaining to CoV	Highly specific for RdRp and N gene	USD 650/100 Reactions
(12)	Allplex™ 2019-nCoV Assay Seegene-Korea [23]	CE-IVD and Korea	It is a multiplex RT-PCR detection method in a single tube	SARS-CoV-2's RdRP, E and N gene and all COV's E gene	Nasopharyngeal and oropharyngeal swabs	1 h and 50 min	Convenient workflow using seegene's technology i.e., automated data analysis	Highly sensitive	USD 5
(13)	GeneXpert® Xpress-Cepheid [24]	EUA	It is Qualitative molecular based RT-PCR detection which accurately differentiate between Flu A, Flu B and SARS-COV-2	N2 gene, RdRp and E gene	Anterior nasal swabs or nasopharyngeal swabs -3 mL	25 min	No special training required Single used, reduced contamination by integrated quality control	Sensitivity 100% and specificity 80%	USD 14.90

FAM fluorescein amidites, *ROX* 6-carboxy-X-rhodamine reference, *MFDS* Ministry of Food and Drug Safety, *EUA* Emergency Use authorization, *CE-IVD* European CE marking for In-vitro diagnostic, *NMRA* National Medicines Regulatory Authority, *FDA* Food and drug administration, *CDSCO* Central Drug Standard Control Organisation, *RUO* Research use only, *SAHPRA* South African Health Products Regulatory Authority, *LOD* Limit of Detection, *ICMR* Indian Council of Medical Research, *HEX* Hexchloro-Fluroscein

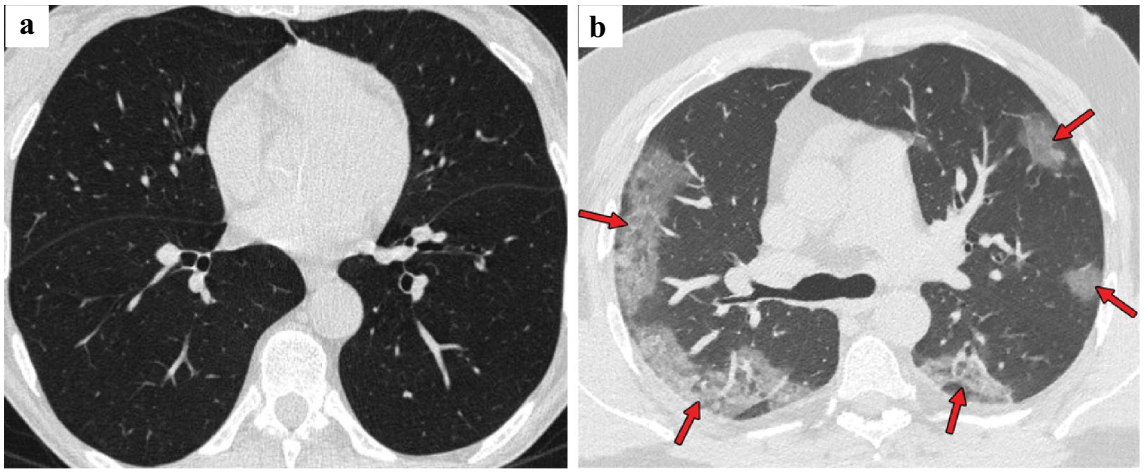

Fig. 2 **a** CT-scan of lungs for a healthy patient [32]. **b** CT-scan image of COVID infected patient with arrows showing ground-glass opacities [32]

radiation for detection and the harmful effects of radiation can prove a big deterrent in using CT-scan imaging for diagnosing COVID-19.

Serological techniques: immunoassay (antibody and antigen detection)

Antigen or antibodies in liquid (generally serum) samples are measured using antigen–antibody interaction. The immune system produces antibodies in response to antigens, such as pathogenic bacteria and viruses. The presence of antibodies (Immunoglobins A, G, and M [IgA, IgG, and IgM]) in body fluid are an indication of the corresponding infection. IgM antibodies corresponding to SARS-CoV-2 are first to be detected since IgM are the first to respond to infection. IgG are detectable 7–10 days after the infection [33].

Antigen-detection diagnostics directly detect SARS-CoV-2 proteins (antigens) present in the human body post-infections. SARS-CoV-2 antigens are replicated in respiratory secretions and nucleocapsid (N) proteins are released in large amounts in serum, fecal matter, urine, and throat wash samples [34]. Diao et al. [35] measured antigen in the infected patients' urine samples, indicating that antigen can also be detected non-invasively. Antigen detection is inexpensive and does not require instrumentation, and its kits can be mass-produced easily. However, it suffers from low sensitivity because of the absence of amplification. Major serological diagnostic kits are compared in Table 2.

Enzyme-linked immunosorbent assay (ELISA)

ELISA is a plate-based assay in which a known antigen or antibody, whose corresponding antibody or antigen, respectively, needs to be detected, is immobilized [54]. During measurements, antigen–antibody complex is formed to which complementary enzyme-linked antigen is bound, followed by the addition of color changing substrate that generates a signal proportional to antigen/antibody concentration. Serum and plasma samples for ELISA are taken by venipuncture and should be done by experts. MacMullan et al. [55] used saliva samples on a commercially available ELISA and detected COVID-19 with 84.2% sensitivity and 100% specificity, indicating saliva as a suitable sample for detection.

Liu et al. [56] and Kohmer et al. [57] observed an increase in the performance of ELISA test from ~ 50 to ~ 80%, when measurements were done between 10 and 18 days instead of 5 and 9 days after the onset of symptoms. ELISA kit developed by Sapkal et al. [58] showed that IgG antibody has the highest sensitivity of 92.37% and reproducibility of 97.9%. My BioSource developed N protein-based ELISA kit costing around USD 600 for 96 tests based on Horseradish Peroxidase (HRP) colorimetric detection system to detect COVID-19 antigens. Che et al. [59] developed monoclonal antibodies-based ELISA to detect N protein with a sensitivity of 50 pg/mL. They also found that the N antigen peaked between 6 and 10 days after the onset of symptoms.

To et al. [60] reported that ELISA is most effective for detecting antibodies of Receptor-Binding Domain (RBD) and N protein of coronavirus. ELISA for the detection of RBD antibodies showed 100% sensitivity for IgG and 94% sensitivity for IgM, whereas N protein antibodies showed lower sensitivity at 94% for IgG and 88% for IgM [33]. After examining the ELISA technique, researchers concluded that it is cheaper than the RT-PCR diagnostic method, has high throughput, but is labor-driven, less sensitive, and hence not suitable for point-of-care detection.

Table 2 Comparison of kits based on serological tests (ELISA, CLIA, and LFIA) for COVID-19 detection

S. No.	Name of the test/kit	Approved/ developed by	Principle	Reagent/biomarkers/ genes targeted	Sample type/sample size of kit	Time of detection/range of detection	Novelty/ other specific information	Accuracy/specificity/ sensitivity/LOD	Cost/sample
ELISA									
(1)	DEIASL019 sars-cov-2 IgG ELISA kit CD creative diagnostics [36]	USA	Qualitative analysis Detection in viral lysate antigen In presence of IgG blue color is emitted by horseradish peroxidase (HRP) linked catalyst	Detected- IgG antibody Substrate—HRP	Serum Plasma	50 min	Total wash step = 2	Specificity 100% and sensitivity 90%	Not available
(2)	COVID-19 IgM ELISA assay kit Eagle Biosciences[37]	Columbia; North America; CE-IVD	Quantitative analysis Change is measured in spectrophotometric microplate reader	Detected—IgM Substrate—HRP, streptavidin	Serum Size = 1 × 96 well plates	Range of detection—20 µl	For research use only	Specificity is determined by cut-off control	USD 785
(3)	EDI COVID-19 Kit Epitope diagnostics Inc. [38]	USA	Qualitative detection of IgM and Quantitative detection of IgG antibodies	Detected- IgG and IgM, full length N gene	Serum	80 min	Total wash step = 2	Limit of detection = 5 iu/mL -	Not available
(4)	COVID-19 IgG coronavirus ELISA Kit My Biosource [39]	Columbia, North America	Quantitative assay Colorimetric technique color change which is measured at 450 nm. The use of HRP to detect the native (not recombinant) covid-19 spike S1 protein subunit capturing antibodies	Detected- IgG antibody Substrate—HRP enzyme For fluorescence—EDTA or Heparin	Serum Plasma homogenate Size = 100 assay per kit	1 h 30 min	For research use only	Highly specific	USD 850/kit
(5)	STANDARQ COVID-19 Ag SD Biosensors [40]	WHO-EUL, Korea MFDS	Qualitative Chromatographic Immunoassay detection of specific antigens to SARS-CoV-2	Detected—Antigen	Nasopharynx swabs Size = 25 tests/kit	15–30 min	Point-of-care testing, Reduced requirement for extra equipment	Specificity 98.94%	Not available
(6)	Platelia SARS-CoV-2 Total Ab Assay BIO-RAD [41]	U.S- EUA, CE-IVD	Semi-quantitative detection of total antibodies of the infected viral genome of SARS-CoV-2 virus. Using ELISA method	Detected—IgG, IgA, IgG in a single assay	Human Serum and Plasma	30 min waiting time for standardization Approx. 1 h	Testing instruments and world-class technical support	Specificity = 99.56% Sensitivity = 92% (100% after 8 days of incubation)	USD 676 for 96 tests

Table 2 (continued)

S. No.	Name of the test/kit	Approved/ developed by	Principle	Reagent/biomarkers/ genes targeted	Sample type/sample size of kit	Time of detection/range of detection	Novelty/ other specific information	Accuracy/specificity/ sensitivity/LOD	Cost/sample
(7)	VITROS Covid-19 Antigen Ortho's Clinical diagnostics [42]	CE-IVD, USAFDA-EUA	In vitro qualitative, immunodiagnostic of the SARS-CoV-2 nucleocapsid antigen	Detected—Nucleocapsid protein	Nasopharyngeal swabs	130 tests/hour	Disposable tips, Intelligent check, Less Manual Error Point-of-care	Highly accurate results are only shown as a YES/NO	Not available
(8)	COVID-19 ELISA kit My Biosource [39]	Columbia, North America	It is based on the ability of the binding the spike protein of SARS-CoV-2 to ACE-2 immobilized in a functional ELISA at 2 µg/mL. (100µL/well) and measured by SDS-Gel electrophoresis	Detected—Antigen Spike S1 protein	Nasopharyngeal swabs and oropharyngeal swabs	Approx 1–2 h	No cross-reactivity	Detection range − 0.391 to 25 ng/mL	USD 445 for 96 tests
CLIA									
(1)	CLIA- SARS-CoV-2 Analyzer Shenzhen YHLO Biotech Co. Ltd [43]	China	Quantitative detection of SARS-CoV-2 viral gene using fluorescent immunochromatography	Detected—Antibody IgG and IgM	Serum Plasma Whole Blood Volume of sample-100uL	3–15 min	Built-in thermal printer, display screen weighs 2.5 kg Point-of-care	Highly specific	Not available
(2)	MAGLUMI 2019-nCoV (SARS-CoV-2) IgM/IgG kits Snibe Diagnostics, China [44]	China CE-IVD	Based on the CLIA Antibody Assay for detection of COVID-19 The antibodies (IgG + IgM) are jointly detected to ensure high clinical sensitivity	Detected- antibody IgG and IgM	Inactivated serum Plasma cells	30 min	Reduce false-negative cases of nucleic acid testing	Highly specific LOD—10 uL	USD 1.4
(3)	LIAISON CLIA-XL SARS-CoV-2 Ag Diasorin S.P.A., Italy [45]	Italy	It is a quantitative assay for detection of N gene of SARS-CoV-2 virus	Detected- Nucleocapsid protein	Serum Plasma	Detection—136 results/hour	Fully automated. Detection within 10 days from symptoms	Sensitivity—97.1% Specificity- 100%	Not available

Table 2 (continued)

S. No.	Name of the test/kit	Approved/ developed by	Principle	Reagent/biomarkers/ genes targeted	Sample type/sample size of kit	Time of detection/range of detection	Novelty/ other specific information	Accuracy/specificity/ sensitivity/LOD	Cost/sample
(4)	SARS-CoV-2 IgM assay on Alinity Abbott Core Laboratories [46]	USA	Based on chemiluminescent microparticle immunoassay (CMIA) which detects IgM antibodies to SARS-CoV-2 quantitatively	Detected- Nucleocapsid protein (N)	Serum Plasma	Detection—4000/24 h	Can't be used as the sole basis of diagnostics	Positive—100% Negative—99.97%	Not available
(5)	ACCEED 260 Chemiluminescence Assay Analyzer Bioscience Ltd. China [47]	China, CFDA—EUO	Magnetic particle chemiluminescence which works on sample clot and, liquid-level detection. Independent high-speed bi-directional automatic mixing is done	Detected- antibodies IgM and IgG	Serum Plasma Size of the analyser—1300 mm × 600 mm × 740 mm	Detection-180Tests/hour	It is a random, batch strong fault handling mechanism	LOD—10–50µL	Not available
LFIA and immunochromatography									
(1)	COVID-19 antigen Access Bio CARESTAR [48]	USA	Detection of SARS-CoV-2 nucleocapsid protein antigen with lateral flow immunoassay. Use to identify mainly acute infection in symptomatic patients	Detected- Nucleocapsid protein	Nasopharyngeal swabs Size- 20 tests/kit	10 min	Point-of-care (POC) designated with a CLIA test	Sensitivity—88.4% Specificity—100%	Price for 1 kit—USD 1.8
(2)	Coronavirus antigen rapid test kit Joysbio [49]	China; CE-IVD	Qualitative detection using lateral flow immunoassay and colloidal gold	Detected—Nucleocapsid protein	Upper respiratory samples Nasal swabs Saliva Size = 1,2,5 and 20 tests/kit	15 min	Less invasive	Accuracy—98.98% LOD – 1.6×10^2 $TCID_{50}$/ml	USD 1.67
(3)	COVID-19 IgG/IgM LFIA Test Advagen Biotech [50]	Brazil	Quantitative detection	Detected- antibodies IgM and IgG	Serum Plasma Whole blood	44 min	Rapid detection	Sensitivity 80% Specificity 100%	Not available
(4)	STANDARD Q COVID-19 Ag SD Biosensors [51]	CE-IVD; WHO; Korea-MFDS	It is a rapid chromatographic immunoassay (LFIA) for the qualitative detection of specific antigens to SARS-CoV-2	Detected antigens	Nasopharyngeal swab	15–30 min Size = 25 tests/kit	no equipment needed, Point-of-care	Sensitivity 94.4% Specificity 100%	Price for 1 kit-USD 1.45

Table 2 (continued)

S. No.	Name of the test/kit	Approved/ developed by	Principle	Reagent/biomarkers/ genes targeted	Sample type/sample size of kit	Time of detection/range of detection	Novelty/ other specific information	Accuracy/specificity/ sensitivity/LOD	Cost/sample
(5)	Sofia SARS antigen Ag FIA-LFIA Quidel [52]	FDA-USA	Qualitative detection using Fluorescent Immunoassay (FIA with lateral flow technology in a sandwich design. It produces automated and objective results	Detected- Nucleocapsid protein (N gene) from SARS-CoV-2	Nasopharyngeal swabs	15 min Size = 25 tests/kit and 12 cassettes	No volume fluctuations, automated tracking, data capture	No cross-reactivity	Not available
(6)	Ag SARS-CoV rapid test Pharm ACT [53]	Germany, CE-IVD	In the lateral flow cassette, a red-pink color front forms and moves across the test strip over a control line (C) and two test lines (IgM and IgG) when the color disappears from one indicates infection	Detected- antigens N protein, and S1 and S2 subunits	Whole blood Serum	20 min	No extra equipment needed	100% with 126 cases	Not available

EUL emergency use listing procedure

Chemiluminescence immunoassay (CLIA)

CLIA's principle is similar to ELISA, but this method uses luminescent chemicals as substrate instead of chromogen [61]. Stationary solid particles are coated with antigen or antibody of interest and light generated upon interaction is proportional to the concentration of analyte [62]. Abbott Core laboratory [8] has provided a CLIA test kit named as 'ARCHITECT-i system', which detects up to 100–200 tests/hour in serum/plasma/whole blood IgG samples and is approved by USA. The main advantage of CLIA is its ability to be unaffected by background signals, thereby giving higher sensitivity.

The use of CLIA for IgG detection showed a sensitivity of 89% and specificity of 91%, whereas for combined IgG and IgM detection, a sensitivity of 97% and specificity of 99% was observed [63]. There are over 100 point-of-care CLIA antibodies detection kits worldwide for COVID-19. Auto bio [64], Shenzhen YHLO [43], and Snibe [43] are some manufacturers from China producing commercial CLIA point-of-care COVID-19 kits. Mesa Biotech [65] and Access bio [48] are companies from the USA developing CLIA-based kits for rapid diagnostics.

Liu et al. [66] developed a nanozyme chemiluminescence portable paper test for rapid and accurate detection of SARS-CoV-2 S antigen. This method, in combination with LFIA, can be used as a novel point-of-care detection method, especially during the early stages of infection. LUMIPULSE and LIASON SARS-CoV-2 CLIA-based antigen detection kits developed by Hirotsua et al. [67] and Dia Sorin et al. [45] validated that antigen test by CLIA shows high sensitivity and specificity toward COVID-19.

Lateral flow immunoassay (LFIA) or immunochromatography: rapid diagnostic tests (RDTs)

LFIA or immunochromatography, commonly called as RDTs, can detect analytes within 5–30 min using capillary action. Dye-labeled antibodies or antigens are captured on nitrocellulose strip to bind complementary antigens or antibodies, showing visible color change for qualitative detection [68]. There are around 400 immunochromatography kits for COVID-19 diagnostic purposes [69].

The SARS-CoV-2 rapid IgG-IgM combined antibody test kit developed in China tested combined IgG and IgM with a sensitivity of 89% and specificity of 91% using antigen RBD as a recognition element [70]. Traugott et al. [71] showed that sensitivity of LFIA kits for antibody detection is based on the duration of symptoms onset (13–20% for < 5 days, 20–80% for 6–10 days, and 100% for > 11 days from symptoms onset). LFIA is cost-effective and rapid but sometimes lacks sensitivity. RDTs for SARS-CoV-2 antigens prefer N protein as its analyte because of its maximum presence. Niclot et al. [72] compared antigen RDT of 138 nasopharyngeal samples to

Table 3 CRISPR-based detection kits for COVID-19

S. No.	Name of the test/kit	Approved/developed by	Principle	Reagent/biomarkers/genes targeted	Sample type/sample size of kit	Time of detection/range of detection	Novelty/other specific information	Accuracy/specificity/sensitivity/LOD	Cost per sample
(1)	iSCAN: RT-LAMP-coupled CRISPR-Cas12 POC Kit KAUST bioengineer (Magdy Mahfouz) [86]	Saudi Arabia	An in vitro Specific CRISPR-based Assay for Nucleic acids detection. The detection depends on the subsequent cleavage of SARS-CoV-2 genomic sequences by the Cas12 enzyme with fluorescent based	E gene with Tris buffer, HEPES buffer, and commercial enzymes (NEB)	Nasopharyngeal swabs	60 min	This assay is suitable for large-scale deployment. Easy to use because the colorimetric reaction coupled to lateral flow immunochromatography	LOD = 10 RNA copies/ reaction	Low cost
(2)	CRISPR/Cas9 POC kit (FNCas9 Editor Limited Uniform Detection Assay) Feluda [81]	TATA group CSIR- IGIB ICMR, India	It is a paper strip based CRISPR-based method. The use of a catalytically inactive FnCas9-gRNA-complex and trans-cleavage activity of reporter molecules like Cas12 or Cas13 methods were used for Cas9 readout results	N gene and S protein	Nasopharyngeal swabs	45 min	Independent of PCR High ease of use Lateral Flow based read out results	Sensitivity = 96% Specificity = 98%	~USD 7
(3)	Sherlock's CRISPR-based assay Sherlock biosciences and Binx health [87]	US-FDA, EUA	It is based on qualitative SHERLOCK- and INSPECTR-based platforms. When the identification occur the CRISPR enzyme activates to release a signal	AapCas12b guide RNAs to target the N gene of SARS-CoV-2	Nasopharyngeal swabs Oropharyngeal swabs Bronchoalveolar lavage	60 min	point-of-care, Home testing platform, hand-held test	Accurate and ability to detect 24 targets at a time Yes/No results	Not available

Table 3 (continued)

S. No.	Name of the test/kit	Approved/developed by	Principle	Reagent/biomarkers/genes targeted	Sample type/sample size of kit	Time of detection/range of detection	Novelty/other specific information	Accuracy/specificity/sensitivity/LOD	Cost per sample
(4)	SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) [83]	USA FDA EUA	This assay includes RT-LAMP based amplification for RNA extraction which is followed by Cas12-based detection which can distinguish the SARS-CoV-2 virus protein	E protein, N protein and human RNAase P gene FAM- biotin reporter	Nasopharyngeal swabs Oropharyngeal swabs	30–40 min	No cross-reactivity Visualized lateral flow strips	Positive predictive = 95% and 100% LOD = 10 copies per µl	Not available

RT-PCR and showed that RDTs have good sensitivity. The Pharmact company has developed a new kit for antigen test named BELMONITOR CoV-2 with high specificity, sensitivity, and accuracy at 99%, 98%, and 98%, respectively, after 7–8 days of infection [73]. Most LFIAs are specific to only one type of antigen, thereby limiting its sensitivity.

Magnetic immunoassay (MIA)

MIA uses magnetic beads as labels to detect a specific analyte. MIA can be conducted in a liquid medium, whereas ELISA and CLIA require a stationary medium [52]. Pietschmann et al. [74] detected SARS-CoV-2 specific antibodies by human serum-based MIA and compared it with ELISA. MIA showed higher sensitivity and a larger detection range with detection time four times faster than ELISA (MIA—42 min; ELISA—161 min). Fabiani et al. [75] developed an immunoassay using magnetic beads to detect spike (S) and N protein of coronavirus with a detection limit of 19 ng/mL and 8 ng/mL, which is comparable to RT-PCR. This method can be easily miniaturized, requires non-invasive samples such as saliva, thus making it an efficient method for commercial use.

Isothermal nucleic acid amplification-based methods

RT-LAMP combines Loop-mediated Isothermal Amplification (LAMP) with reverse transcription to detect RNA at a lower cost to RT-PCR [76]. The target sequence is amplified at isothermal condition (60–65 °C) and polymerase is added along with primers, which has both replication and strand displacement activity [77]. In the isothermal process, DNA strands are not denatured by heat, thereby increasing the amount of DNA produced. Amplification products can be easily detected by simple photometry, replacing the need of complex instrumentation [78]. Thi et al. [79] collected several hundred clinical RNA pharyngeal swabs samples from individuals tested for COVID-19 and confirmed that RT-LAMP assay was simpler compared to RT-PCR for large-scale testing of SARS-CoV-2. Park et al. [80] observed that RT-LAMP can detect as low as 100 copies of SARS-CoV-2 RNA with no cross-reactivity to other human coronaviruses.

Emerging diagnostic technologies and devices

CRISPR (clustered regularly interspaced short palindromic repeats)

Cas9 is CRISPR-associated protein 9 that serves as an enzyme which uses CRISPR sequences to recognize specific

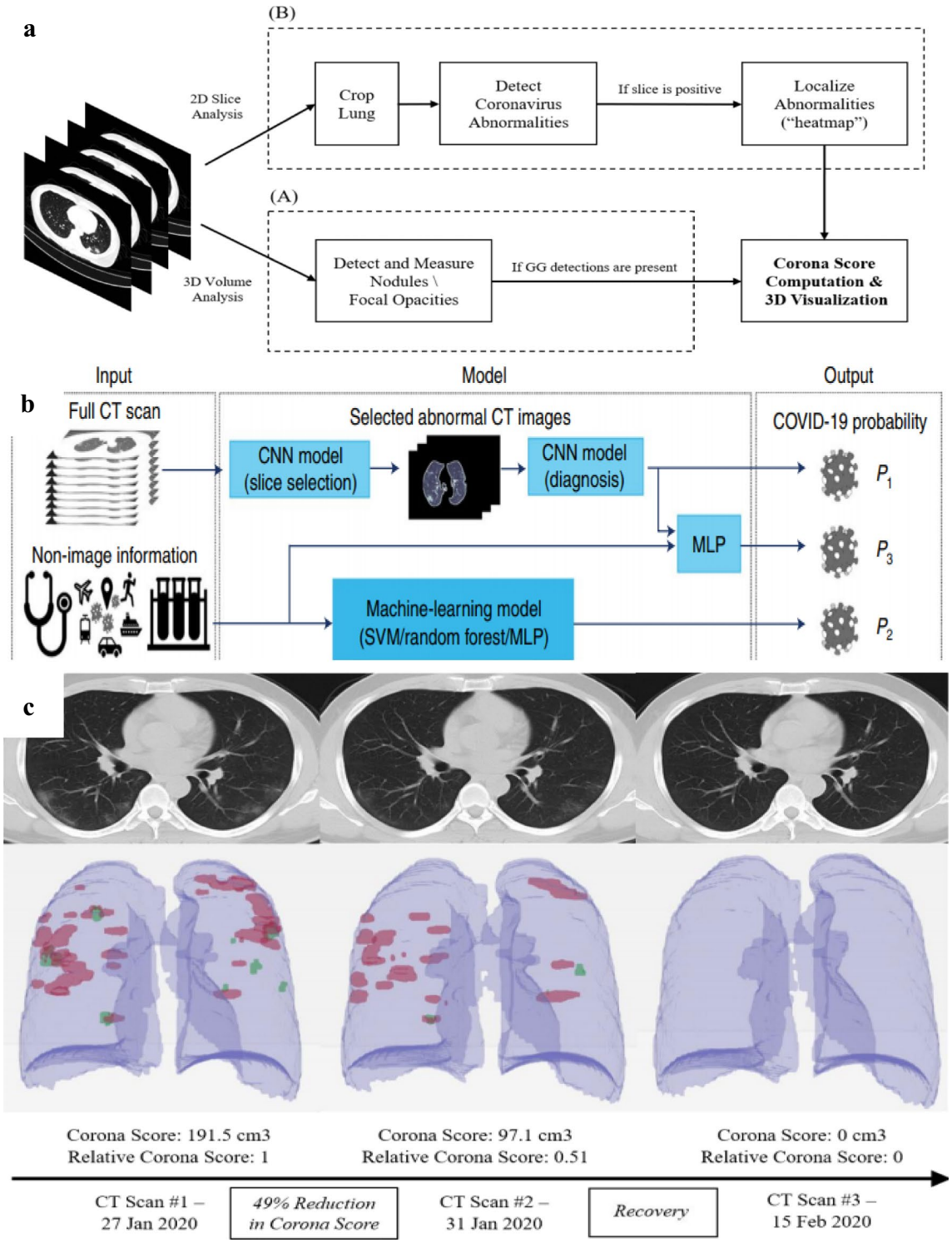

Fig. 3 **a** Block diagram for identifying infection from CT scans. **b** Illustration of the framework used for modeling. **c** Corona score calculated from the model for patients at different levels of infections [89]

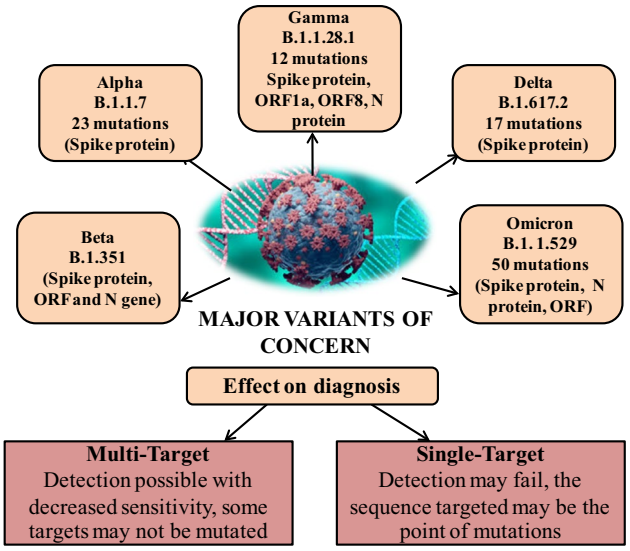

Fig. 4 Variants and its effects on diagnosis [100, 101]

strands of DNA and cleave them [81, 82]. DETECTR, developed by Chen et al. [83], activates Cas12a after binding with SARS-CoV-2-cDNA. It further cuts specific labeled probes, confirming the presence of the virus. Wang et al. [84] proposed an ultrasensitive visual SARS-CoV-2 detection using integrated RT-LAMP and CRISPR/Cas cleavage in one pot within 45 min. It showed 100% positive predictive agreement. Huang et al. [85] developed an assay utilizing custom CRISPR Cas12a/gRNA complex to detect target amplicons generated during RT-PCR within 50 min with a detection limit of 2 copies/sample. Other CRISPR-based nucleases such as fnCAS9 and Cas12a have also been used for COVID-19 detection, exhibiting high specificity and faster detection [86]. Sherlock Biosciences [87] received approval from US FDA for employing CRISPR-based diagnostic kits for screening. Some of the CRISPR kits available for use and in the research stage are discussed in Table 3.

Artificial intelligence (AI)

Machine learning-based screening of SARS-CoV-2 can use data from the large-scale screening of COVD-19 patients and evaluated using neural network classifiers [88]. Similarly, a deep learning-based analysis system using thoracic CT images as input was constructed for automated detection and monitoring of COVID-19 patients over the time of infection. Multiple images of CT were first provided to CNN (Convolution Neural Network) to predict the probability of the infection. Ground-glass Opacities gives the final detection and visualization, confirming infection of COVID-19 giving a corona score of the infected patient based on the level of infection. Figure 3 illustrates the framework used for

Worst → Best

Name of technique	Clinical Use	Effect of mutation	Accuracy	Infrastructure	Detection time	LOD	Qualitative/ Quantitative	Time required after infection for detection
RT-PCR	Most-used method	Minimal effect (for multi-target)	High	Laboratory required	4-5 hrs	Low LOD	Both	1-8 days
CT-Scan	Secondary to RT-PCR	Moderate (vary with symptoms)	High, if lung is infected	Complex & expensive	4-5 hrs	Only lung infection	Both	4-15 days
ELISA	For antibody analysis	Moderate	Moderate to high	Laboratory required	1-2 hrs	Moderate LOD	Both	8-15 days
LFIA	Rapid antigen test	Moderate	Moderate to high	Point-of-care device	10-20 mins	Moderate LOD	Only qualitative	After 11 days
MIA	No (Research stage)	Moderate	Moderate to high	Minimum required	10-15 mins	Lower than ELISA	Both	After 15 days
CRISPR	Moderate use	Minimal effect (for multi-target)	High	Laboratory required	40-60 mins	Low LOD	Both	5-10 days
Micro array	No (Research stage)	Can identify variants	High	Minimum required	2-3 hrs	Low LOD	Only quantitative	5-10 days
Bio-sensors	No (Research stage)	Moderate	Moderate	Point-of-care devices	Within 10 mins	Moderate LOD	Both	10-15 days
RT-LAMP	Small use	Minimal effect (for multi-target)	High	Laboratory required	1-2 hrs	Lower than RT-PCR	Both	1-8 days

Fig. 5 A comparative analysis of different methods used for the diagnosis of coronavirus

modeling clinical data (CT Scan Images). These automated diagnostic systems can be used as efficient methods to detect COVID-19 [89, 90].

Microarray

Multiple DNA samples are used to construct an array and expression is indicated by the amount of mRNA bound to each site. Data are collected and profile for gene expression is generated. Parallel analysis of genes can simultaneously provide information on thousands of genes, an advantage of this method [91]. The advent of many different variants of coronaviruses and increased infections and virulence of the new variants make DNA Microarray an effective diagnostic technique in detecting COVID-19.

Hedde et al. [92] used coronavirus antigen microarray (CoVAM) to measure antibody levels in serum samples from 23 strains against 67 antigens of 10 viruses known to cause respiratory tract infections, including SARS-CoV-2. Detecting a large number of antigens simultaneously in a single test considerably increases specificity and sensitivity. CoVAM is a robust and 3D-printable portable imaging platform that can be deployed immediately with minimal infrastructure at the cost of ~ USD200 per unit. Samples include a few drops of blood from a finger prick to determine the presence of antibodies to SARS-CoV-2 with a test turnaround time of 2–4 h. Assis et al. [93] suggested that CoVAM could be used both as an epidemiologic and research tool.

Nanotechnology

There has been considerable use of silver nanoparticles (AuNPs) as immobilizers in LFIA and CLIA [94, 95]. Zhao et al. [96] have developed a one-step method using pre-coated metallic nanoparticles for viral RNA extraction of COVID-19, a one-step method combining lysis and binding. Pre-coated metal nanoparticles–RNA complexes can be directly added into subsequent RT-PCR reactions completing purification within 20 min. Chandra et al. [97] developed point-of-care immunosensors for detecting antigens based on electrochemical nano-dendroids and graphene oxide nanocomposites. Martens et al. [98] developed COVID-19 antigen respi-Strip, point-of-care device based on membrane technology with colloidal gold nanoparticles. Sensitivity and specificity were recorded as 99.5% and 57.6%, respectively, with an accuracy of 82.6%. Verma et al. [99] showed that gold nanoparticle-coated peptides could be used as a rapid-detection tool for COVID-19. All these emerging techniques may prove highly useful for developing point-of-care devices.

Mutations in coronavirus and its effect on diagnosis

Changes in S, N, membrane (M), and envelope (E) proteins lead to different variants of virus. Some variants of concern are Alpha, Beta, Delta, and Gamma, with Omicron being the newest variant. It has ~ 30 mutations having multiple effects on the human body. Different variants of SARS-CoV-2 can affect diagnosis performance. Tests that have antibodies as recognition elements can fail because of a change in antigens' structure, rendering them useless for detection. Changes have been largely observed on S protein of coronavirus; hence, immunoassays focusing on detecting N protein and antibodies corresponding to N protein might remain unaffected in detecting different variants in comparison to detections based on S protein [100].

Molecular tests, such as RT-PCR, RT-LAMP, and Microarray, have less chances of failing, since molecular diagnosis focus on multiple targets. Other sequences targeted that are void of any mutation can still give satisfactory results. Sensitivity of the diagnoses has been shown to get affected, both for molecular diagnosis as well as point-of-care serological techniques. Even for multi-target diagnostic techniques, overall sensitivity and performance is reduced (Fig. 4) [101]. Deletion of nine nucleotide sequences in N protein in new variant, Omicron has impacted diagnosis significantly. Many RT-PCR kits however target only single gene site; hence, there is a possibility that deleted N-protein portion (in Omicron variant) is the one targeted by the currently available diagnostic method. Hence, molecular testing devices which focus on a single target can fail in the face of emerging variants.

Conclusions and future perspectives

This review covers different diagnostic techniques currently in use in various clinical setups to diagnose COVID-19. RT-PCR is considered as a gold standard method for diagnosis, and all other measurement techniques are compared, taking RT-PCR as a reference, in terms of their measurement principle, sensitivity, accuracy, and cost. RT-PCR focuses on measuring nucleic acids for diagnosis of infection, whereas a wide range of measurement techniques are based on serological tests. Both antigen and antibody are detected in serological tests. The most favorable time for antibody measurements is 14 days after the onset of symptoms to up to 60 days, while antigen detection can be used for early detection starting from the second day of infection to 14 days. The detection of antigen in blood by LFIA can provide an efficient point-of-care diagnostic technique for fast detection. Some rapid diagnostic tests (RDTs) have been developed

with limited accuracy, and further advances in this field can lead to the development of highly sensitive and accurate point-of-care testing devices. Technologies in the research phase, such as developing electrochemical biosensors and AI to provide insights from data, can prove useful for fast and accurate detection. A comprehensive comparison of all methods is presented in Fig. 5.

It has been observed that even in the wake of different mutations of coronavirus, RT-PCR detecting multiple targets may still be efficient and give accurate results (with lower sensitivity) for new variants. However, RT-PCR has limitations such as the requirement of laboratory facilities, complex preparation and detection steps. To overcome these limitations, other nucleic acid methods such as RT-LAMP and microarray can be used to develop fast and efficient detection methods at a low cost. MIA presents major benefits over ELISA with a lower detection limit, lower detection time, and fewer intermediate steps. MIA can be developed for immunoassay detection over ELISA and CLIA with considerable benefits.

A lot of research is underway for the development of accurate methods. However, more rigorous studies are needed in transferring lab-based methods to clinical trials. Efforts with the right emphasis and providing essential tools to the research community can lead to the development of effective point-of-care diagnosis with minimum sample preparation steps, lower detection time, and better accessibility.

Acknowledgements The author Shagun Sharma would like to acknowledge KARYA (Knowledge Augmentation through Research in Young Aspirants) Program under the Department of Science and Technology (DST)—Government of Rajasthan, which provided research opportunity under the mentorship of Indian National Young Academy of Sciences (INYAS) and Tata Consultancy Services (TCS) Research.

Funding This work was funded by TCS Research, Tata Consultancy Services Ltd. (IN).

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical standards The manuscript does not contain clinical studies or patient data.

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