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Review Article

SARS-CoV-2/COVID-19 laboratory biosafety practices and current molecular diagnostic tools

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ABSTRACT

The ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/coronavirus disease 2019 (COVID-19) pandemic has crippled several countries across the globe posing a serious global public health challenge. Despite the massive rollout of vaccines, molecular diagnosis remains the most important method for timely isolation, diagnosis, and control of COVID-19. Several molecular diagnostic tools have been developed since the beginning of the pandemic with some even gaining emergency use authorization from the United States (US) Food and Drug Administration for *in vitro* diagnosis of SARS-CoV-2. Herein, we discuss the working principles of some commonly used molecular diagnostic tools for SARS-CoV-2 including nucleic acid amplification tests, isothermal amplification tests, and rapid diagnostic tests. To ensure successful detection while minimizing the risk of cross-infection and misdiagnosis when using these diagnostic tools, laboratories should adhere to proper biosafety practices. Hence, we also present the common biosafety practices that may ensure the successful detection of SARS-CoV-2 from specimens while protecting laboratory workers and non-suspecting individuals from being infected. From this review article, it is clear that the SARS-CoV-2 pandemic has led to an increase in molecular diagnostic tools and the formation of new biosafety protocols that may be important for future and ongoing outbreaks.

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) was declared a public health emergency by the World Health Organization (WHO) on January 30, 2020. As of September 22, 2021, the virus still continues to spread globally with 229,373,963 confirmed cases of COVID-19, including 4,705,111 deaths being reported to the WHO [1]. Despite massive rollout of vaccines to control and reduce these numbers, challenges remain, including the emergence of SARS-COV-2 variants that reduce vaccination efficacy, vaccinating everyone in a timely manner, and the laxity of individuals/nations to accept vaccination [2–4]. This means that diagnosis still plays a key role in early detection and timely isolation of infected individuals to control further spread.

Among the most commonly used diagnosis methods, nucleic acid amplification tests (NAATs) such as reverse transcription polymerase chain reaction (RT-PCR), remain the gold standard for COVID-19 diagnosis. Despite its efficacy, reports have suggested that RT-PCR has some limitations including the need for tight control to avoid cross-contamination and the multi-step procedure [5–7]. Proper detection procedures must be followed to avoid false negative or false positive results especially in asymptomatic patients or patients with a low viral load [8–10]. Alternative diagnostic methods such as antigen strips have also been used to detect SARS-CoV-2 from COVID-19 samples. The emergence of this pandemic has exposed the limitations of several techniques, while also leading to the development and discovery of new molecular techniques that will help in the diagnosis of both the current and future pandemics. The United States (US) Food and Drug Administration (FDA) has reviewed several molecular diagnostic tests and instruments for the diagnosis of COVID-19 and have granted them emergency use authorization (EUA) [11,12]. These molecular tests can be generally classified into NAATs, rapid diagnostic tests (RDTs), point of care tests (POCs), serological tests, and isothermal amplification tests (IATs). Apart from test assays, new instruments have also been developed to control the pandemic and existing equipment (e.g., Xpert) has been repurposed to detect SARS-CoV-2.

Aside from misdiagnosis, laboratory personnel and unsuspecting individuals may be at risk of COVID-19 infection if individuals do not follow proper biosafety practices [13,14]. To ensure safe detection, proper biosafety practices should be observed not only during detection but also during sample collection, transport, and processing [8]. The WHO and Centers for Disease Control (CDC) in different countries have drafted laboratory guidelines to assist laboratory personnel during COVID-19 diagnosis [15–17]. In this review, we comb through the literature to highlight some of these biosafety protocols while also giving an update on current molecular diagnosis tools for COVID-19, their working principles, and examples of tests that have gained US FDA-EUA.

2. Biosafety considerations during the detection process of SARS-CoV-2/COVID-19

Detection of SARS-CoV-2 or clinical samples from COVID-19 patients should be performed in appropriately-equipped laboratories by well-trained personnel using relevant technical and biosafety procedures. Practicing and observing proper biosafety from SARS-CoV-2 sample collection to detection is essential for the successful and accurate diagnosis of COVID-19 [18]. Before testing, laboratories should perform a site-specific and activity-specific risk assessment and follow standard precautions when handling SARS-CoV-2/COVID-19 specimens. Internationally, four biosafety levels (BSL-1 to 4) exist. Each level has its own rules and practices and the rules become more stringent with increased biosafety level. Descriptions of these biosafety levels, practices, and pathogens are provided in detail elsewhere [17]. For SARS-CoV-2 and COVID-19, the WHO and CDC have outlined specific guidelines to assist in COVID-19 laboratory biosafety [15–17]. Using these guidelines, we have summarized common biosafety practices necessary for successful detection of SARS-CoV-2/COVID-19, as shown in Table 1. In the event that a laboratory cannot meet the required biosafety recommendations, it should consider transferring the SARS-CoV-2/COVID-19 specimens to regional, national, or even international reference laboratories with SARS-CoV-2/COVID-19 detection capacity that fulfill the biosafety requirements.

3. Molecular diagnostic tools for SARS-CoV-2/COVID-19 detection

Since the beginning of the pandemic, scientists and technicians have worked on all fronts to develop new techniques and assays capable of the sensitive detection of SARS-CoV-2. From these advances, several companies have filed for US FDA-EUA approval. As summarized in Table 2, the total number of approved *in vitro* SARS-CoV-2 diagnostic tests by the US FDA includes 262 molecular tests, 32 antigen-based tests, and 88 serology and other adaptive immune response tests. In this section, we focus on some of these molecular diagnostic tests including NAATs, RDTs, and IATs.

3.1. Nucleic acid amplification-based tests (NAATs)

NAATs are the method of choice for confirming the presence of SARS-CoV-2 in samples including COVID-19 samples. Several NAATs exist and they are principally designed to detect genetic material (nucleic acids). Briefly, once the genetic material of a specimen is obtained, it is amplified. This amplification aids NAATs to detect small amounts of RNA in specimens leading to increased sensitivity [19]. The WHO and CDC recommend that NAATs be used on upper and lower respiratory tract specimens [19]. Since the outbreak of COVID-19, many NAATs have gained EUA by organizations such as the US FDA. Below we summarize the working principle of some of these NAATs including RT-PCR and IATs:

3.1.1. RT-PCR

RT-PCR is the current gold standard detection method for confirmation of COVID-19. Of all the NAATs, real-time reverse transcription quantitative PCR (RT-qPCR) is the most commonly used method for the detection of SARS-CoV-2 and confirmation of COVID-19 [20]. RT-PCR greatly relies on primers and/or probes for detection and since the outbreak, several primer and probe sets have been designed for COVID-19 diagnosis and their sensitivity has been determined [21–23]. These primer and probe sets target various regions of SARS-CoV-2 including the nucleocapsid (N), envelope (E), and spike (S) genes, as well as other open reading frames (ORFs) [22]. All US FDA-EUA approved RT-PCR test kits (Table 2) must include two or more of these targets using multiplex RT-PCR protocols for detection. Briefly, as shown in Fig. 1, after collection and inactivation, the sample is lysed (to expose its RNA) and extracted (to purify it and remove potential RT-PCR inhibitors) [24]. Lysis/extraction is performed using commercial kits either manually or automatically. Subsequently, the purified RNA containing both human genetic material and SARS-CoV-2 viral RNA, is amplified and detected by RT-PCR. It is important to note that after RNA is generated, it can first be reverse transcribed to complementary DNA (cDNA) and used as a template for PCR (two-step RT-PCR) or it can be directly used as a template for PCR (one-step RT-PCR). Either cDNA or RNA can be used for detection by RT-qPCR and reverse transcription droplet digital PCR (RT-ddPCR).

Table 1
Summary of the WHO and CDC laboratory biosafety guidelines for SARS-CoV-2/COVID-19 detection [15–17].

Key points/steps	Example	Biosafety notes/precautions	PPE
General	SARS-CoV-2 is easily transmitted through the respiratory tract	Site-specific and activity-specific risk assessment should be performed before any collection or test following standard procedures for SARS-CoV-2/COVID-19 samples; Tests to be done with personnel demonstrating competency to perform the tests in strict adherence to any relevant protocols stated at all times	Dependent on step
Sample collection	Upper respiratory tract	Droplet precautions for common procedures like pharyngeal swab collection; airborne precautions for collections of specimens like nasopharyngeal aspirate/wash, sputum, tracheal aspirate, pleural fluid and bronchoalveolar lavage fluid	Determined after risk assessment. Generally; Medical mask/fit-tested respirators, disposable gloves, gowns*
	Self-collection	Wash hand with soap before collection; collect specimen as directed exactly; send samples as soon as possible as directed by manufacturer's instructions	
	Environmental	Risk assessment should be conducted prior to collection; virus concentration procedures to be done in a BSL-2 facility with unidirectional airflow and BSL-3 precautions; propagative tests like culture to determine infectivity to be done in a BSL-3 facility	
	POC, near-POC, and RDTs	Each test has a specific type of specimen and should be collected as stated in assay IFU (e.g. serum/saliva for serological tests); Person collecting specimen to be 6 feet away from patient, maintain proper infection control wearing appropriate PPE	
Transport and shipping	Short distance	Specimens from suspect or confirmed COVID-19 cases can be transported in sealed biohazard labeled zip-lock bags or containers within a leak-proof cryobox [18]	*
	Long distance (packaging and shipping)	Specimens from suspect or confirmed COVID-19 cases to be transported as UN3373, "Biological Substance Category B" and SARS-CoV-2 viral cultures or isolates to be transported as Category A, UN2814, "infectious substance, affecting humans"	
Sample processing	Initial processing and inactivation	Before inactivation, samples should be opened in a validated BSC or primary containment device; Specimens should preferably be well labelled, in a leak-proof container, and test to be done noted; sample inactivation should be done in a BSL-2 facility with unidirectional airflow and BSL-3 precautions after proper risk assessment; Manufacturer's instructions should be followed where possible	BSL-3 PPE and precautions during inactivation
	Extraction, reagent preparation, and amplification	These three steps should be done in separate rooms; samples should flow in a unidirectional manner to avoid contamination that may lead to false negative results	After inactivation; masks, disposable gloves, gown
POC, near-POC, and RDTs	Ag-RDTs, Ab-RDTs, LAMP	Risk assessment should be conducted and proper precautions be set; Tests can be performed in a normal bench without using a BSC on large paper towel in a well-ventilated area (otherwise use respirators) free of clutter and with no personal stuff, documents or computers; Follow manufacturer's instructions for performing tests and decontamination after testing as specified exactly; Appropriate PPE should be worn	*
Propagative	Culture and neutralization assays	Should be done in BSL-3 laboratories following BSL-3 practices	BSL-3 PPE
Animal experiments	Inoculation for SARS-CoV-2 recovery	All experiments involving animals should be done in ABSL-3 prior following ABSL-3 rules prior to testing in lower laboratories e.g. BSL-2	ABSL-3 PPE
Disinfectants	Alcohol, hypochlorite, chloroxylenol, povidone-iodine, and benzalkonium chloride	Disinfectants proven to be active against enveloped viruses are active against SARS-CoV-2 when used according to manufacturer's recommendations; After selecting disinfectants, attention should also be paid to contact time, dilution, shelf-life and expiry date once working solutions are prepared	Dependent on step during application
Decontamination and waste management	Surfaces, used materials etc.	Known to be, or potentially to be contaminated surfaces or materials by biological agents during work should be properly disinfected; Identify and segregate wastes properly before decontamination; If not done in the laboratory, or on-site, package contaminated waste in a leakproof bags before transfer/transport to another facility capable of decontaminating the waste	Dependent on step during application

SARS-CoV-2/COVID-19 – Severe acute respiratory syndrome coronavirus 2/coronavirus disease 2019; WHO – World Health Organization; CDC – Centers for Disease Control; BSL – Biosafety level; BSC – Biosafety cabinet; POC – Point of care; RDT – Rapid diagnostic test; IFU – Information for user; ppm – Parts per million.
* Specific type to be chosen after proper site-specific and activity-specific risk assessment.

3.1.1.1. RT-qPCR. After extracting the RNA and obtaining the template cDNA, SARS-CoV-2 targets are amplified through cycles of denaturation, annealing, and extension using probe-based (e.g., TaqMan) or intercalating (e.g., SYBR green) dyes that attach to the double-stranded DNA (dsDNA). In particular, the DNA polymerase exonuclease activity cleaves probes/dyes annealed to the specific SARS-CoV-2 targets to exhibit increased fluorescence that can be captured as real-time fluorescent signals on a monitor [7,24–26] (Fig. 1B). These signals are converted to qualitative cycle threshold (CT) values or concentration values in copies/μL through relative quantification. Since RT-qPCR cannot quantify SARS-CoV-2 targets directly, a reference sample of known concentration is needed to develop a standard curve for relative quantification. This is one of the limitations of RT-qPCR compared with RT-ddPCR. So far, none of the US FDA-EUA approved kits can perform relative

Table 2
Examples of recently approved *in vitro* molecular diagnostic tests for SARS-CoV-2 by the US FDA [11].

Test	Totally approved*	Method	Example#						
			Assay name	Specimen	SARS-CoV-2 target	Detection Instrument	Assay time	Setting	LoD
RT-qPCR	207	RT-PCR	cobas SARS-CoV-2	ANS, NS, ANS, NPS, OPS	ORF1a/b, E	Cobas (6800/8800)	~2h 30 mins	H, M, H-Pooling	46 cp/ml
RT-ddPCR	3	RT-PCR	Bio-Rad SARS-CoV-2 ddPCR Kit	NPS, ANS, MNS, NPW/A	N1, N2	QX200, QXDx	6.6 h/96 samples	H	625 cp/ml
RT-LAMP	9	IAT	Lucira CHECK-IT COVID-19 Test Kit	ANS	N	Lucira (colorimetric)	30 mins/ sample	Home, H, M, W	2700 cp/swab
RT-LAMP, CRISPR	2	IAT	Sherlock CRISPR SARS-CoV-2 Kit	NS, NPS, OPS, NPW/A, NA, BALF	ORF1ab, N	Microplate reader (fluorometric)	1 h/run	H	6750 cp/ml
TMA	7	IAT	Aptima SARS-CoV-2 assay	NS, NPS, OPS, MNS, NPW, NPA, NA	ORF1ab	Panther fusion (chemiluminescent)	2.4 h/ run	H, pooling	0.026 $TCID_{50}$/ml
NEAR	1	IAT	ID NOW COVID-19	NS, NPS, OPS	RdRp	ID NOW (fluorometric)	13 min/ run	H, M, W	125 cp/ml
RT-HDA	1	IAT	Solana SARS-CoV-2 Assay	NPS, NS	pp1ab	Solana (fluorometric)	30 mins (12 samples)	H, M	11,600 cp/mL
Sequencing	6	NGS	SARS-CoV-2 NGS Assay	NPS, OPS, MNS, ANS, NS/A, NPW/A, BALF	Entire viral genome	Illumina NextSeq (500/550/550Dx)	~ 12 h	H	800 cp/ml
Ag-based immunoassays	32	Ag-RDT (e.g. LFIA), ELISA, CLIA	CareStart COVID-19 Antigen Home Test	ANS	N	LFIA strip (visual readout)	10–15 mins	Home, H, M, W	2800 $TCID_{50}$/ml
Ab-based immunoassays	88	Ab-RDT (e.g. LFIA), ELISA, CLIA	ADVIA Centaur SARS-CoV-2 Total (COV2T)	Plasma, serum	Total antibody (Including IgG, IgM)	ADVIA Centaur XP (chemiluminescence)	10–15 min	H, M	0.5 index

SARS-CoV-2/COVID-19 – severe acute respiratory syndrome coronavirus 2/coronavirus disease 2019; N(A/S) – Nasal (aspirate/swab); NP(S/W/A) – Nasopharyngeal (swab/wash/aspirate); BALF – Bronchoalveolar fluid; ANS – Anterior nasal swab; MNS – Mid-turbine nasal swab; OPS – Oropharyngeal swab; LoD – Limit of detection; pp1ab - SARS-CoV-2 non-structural polyprotein; NGS – Next generation sequencing; HDA - Helicase-dependent amplification; CLIA – Chemiluminescence immune assay; ELISA – Enzyme linked immunosorbent assay; LFIA – Lateral flow immunoassay; Ag – Antigen; Ab – Antibody.
* Total number was obtained by entering keywords for the diagnostic tests and methods into the US FDA website search tool online [11,12].
#A representative example of the details of how the approved kits work, this does not apply to other similar assays.
H – Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.
M – Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate complexity tests.
W – Patient care settings operating under a CLIA Certificate of Waiver.

quantification as they are designed for qualitative analysis based on resultant CT values. The whole analytical process from extraction to detection by RT-qPCR takes approximately 4–5 h depending on the protocol used [7]. This ensures that results can be reported in a timely manner and positive COVID-19 patients can be quarantined to reduce further spread. Despite its wide use and merits, RT-qPCR has some limitations including: the occurrence of false positive and false negative results when detecting low viral load samples, low tolerance to inhibitors present in samples, contamination from operators, and pre-analytical and analytical bias arising from sample collection, transport, storage, and handling [5,7,24,25,27]. Hence, one needs to take care when handling RT-qPCR samples and ensure proper biosafety measures, such as changing gloves and working in biosafety cabinets when performing RT-qPCR.

3.1.1.2. RT-ddPCR. Droplet digital PCR (ddPCR) is the third generation of PCR that was designed to overcome the limitations of qPCR in absolute quantification of nucleic acid targets [28–31]. ddPCR can directly quantify targets without the need for a standard curve. Compared with RT-qPCR, RT-ddPCR has several advantages including higher sensitivity when detecting low viral load samples, high tolerance to inhibitors, and lower limits of detection [27,32–34]. The general workflow from sample collection to extraction and generation of cDNA (two-step) is similar to that of RT-qPCR.

However, after generating the RNA or cDNA template, in RT-ddPCR (Fig. 1A), the reaction mix is first distributed randomly into thousands of millions of nanoliter-sized droplets by a droplet generator using water-in-oil emulsion technology and microfluidics [35]. This ensures that some droplets will contain one or more SARS-CoV-2 targets, while others will have no targets. These droplets are then transferred to a thermal cycler for PCR amplification to end-point. PCR-amplification to end-point is thought to increase the sensitivity of RT-ddPCR. The amplification process of RT-ddPCR is similar to that of RT-qPCR with no real-time fluorescence data. Unlike RT-qPCR where amplification occurs in bulk, in ddPCR, the amplification process (including annealing, denaturation, and extension) occurs in discrete droplets. Post amplification, the droplets are read on a droplet reader, where positive droplets exhibit increased fluorescence and negative droplets show no fluorescent signal. Using Poisson statistics, the concentration of these droplets is determined by copy number and used for data analysis. The entire process from COVID-19 sample processing to detection may take 6–8 h. Some of the limitations of this method that affect its wide adaptability include cost, availability, complexity, and

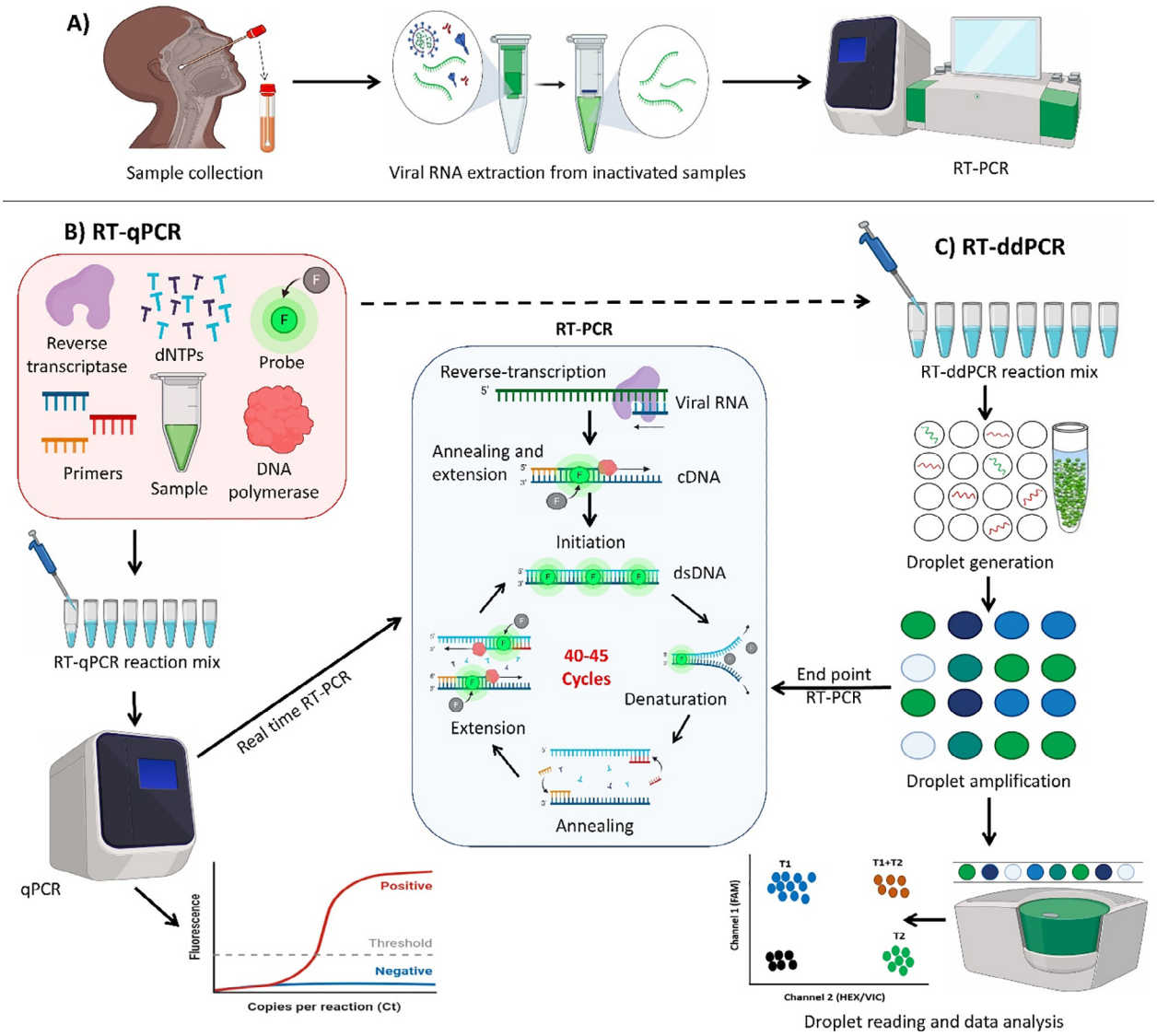

Fig. 1. RT-PCR analysis of SARS-CoV-2/COVID-19 samples. A) General workflow including sample collection to detection by RT-PCR. B) RT-qPCR detection workflow. C) RT-ddPCR detection workflow.

time. To date, only three SARS-CoV-2 RT-ddPCR kits have being approved by the US FDA-EUA, as summarized in Table 2. Owing to its advantages, more should be done to include RT-ddPCR in routine diagnostic procedures. Its ability to perform absolute quantification can be leveraged to generate reference standards for validation of other SARS-CoV-2 NAATs [36].

3.1.1.3. Rapid RT-PCR-based diagnostic tests. As suggested by their name, RDTs that are based on the principle of RT-PCR also exist. Principally, RDTs are designed to have less hands-on time and deliver results rapidly while maintaining a level of sensitivity similar to or better than other NAATs [24]. An example of an RDT is the US FDA-EUA approved pert Xpress SARS-CoV-2 assay. In addition to test kits, devices have also been optimized to used RT-PCR based on microfluidics to detect SARS-CoV-2. For example, Wu *et al.* combined RT-dPCR and CRISPR to form a RApid DIgital Crispr Approach (RADICA) for absolute quantification of nucleic acids in 40–60 min [37] and Yin *et al.* combined RT-ddPCR with rapid PCR to achieve ultrafast detection of SARS-CoV-2 within 7 min with similar or better detection accuracy than RT-qPCR [38].

3.1.2. Isothermal amplification tests (IATs)

Unlike RT-PCR where amplification of nucleic acid targets occurs through cycling of temperatures, IATs use a constant temperature to amplify nucleic acid targets, as summarized in Fig. 2. Such tests are therefore rapid (owing to the elimination of PCR cycles), portable, and relatively cheap (owing to the elimination of expensive equipment, such as thermal cyclers) [7,24,39]. Compared with RT-PCR, IATs are simple and easy to operate, hence some can be used at home or at POC by doctors, nurses, or clinicians with minimal experience with NAATs by following the kit protocol in detail. Principally, IATs work by denaturing nucleic acids either thermally or enzymatically and then amplifying the targets [7,24,25,39]. The majority of IATs were previously used for the detection of DNA; however, in response to SARS-CoV-2, these tests have been tweaked by introducing a reverse transcription (RT) step [39]. Currently, several IATs have been used to detect SARS-CoV-2, as summarized in Table 3, and their working principles have been reviewed in detail elsewhere [7,24,39–41]. Despite their wide availability, EUA of IATs is still lagging behind that of RT-PCR largely due to sensitivity and specificity issues associated with IATs. Regardless, IATs still play an important role in COVID-

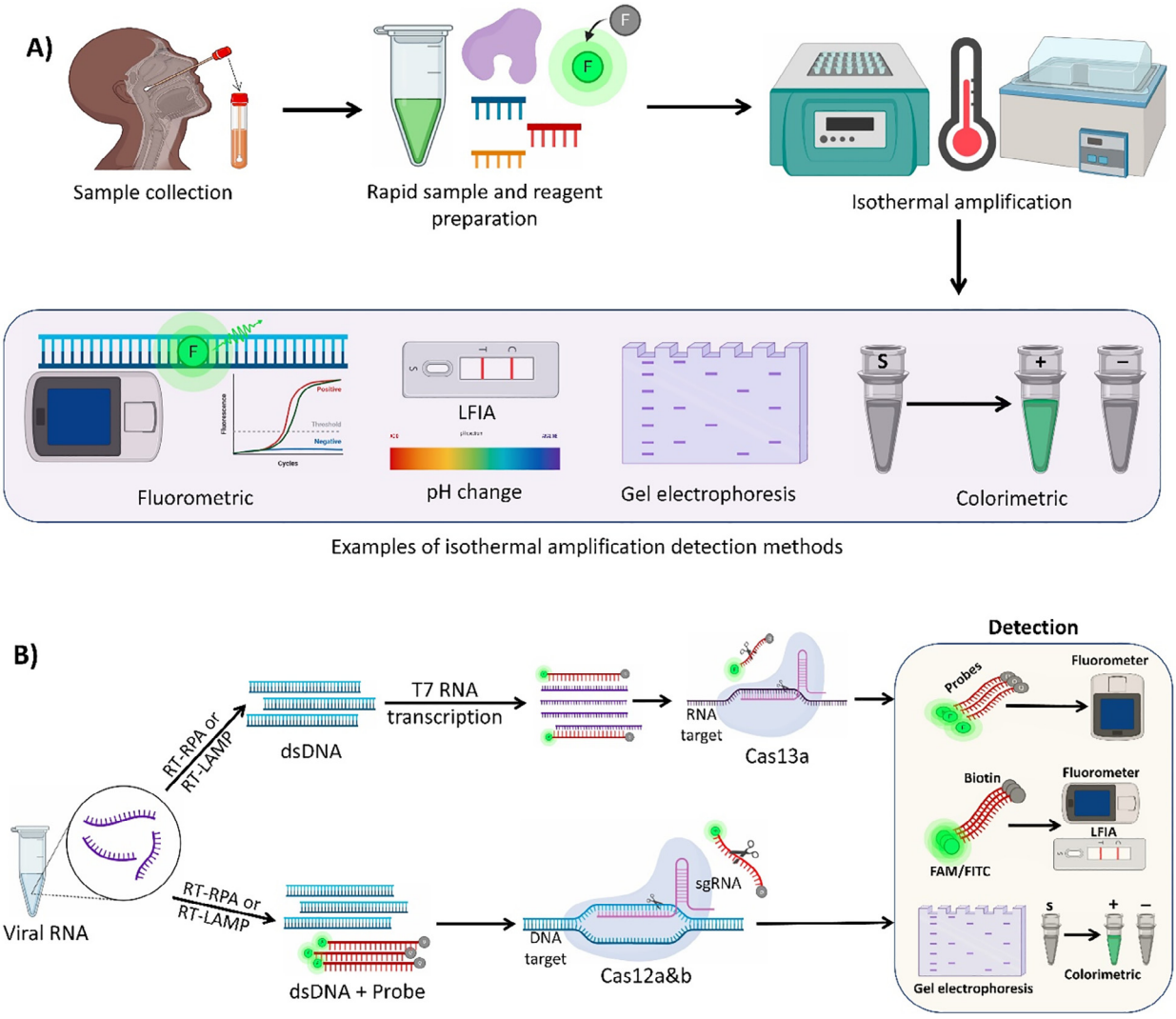

Fig. 2. IAT detection workflow. A) The general workflow for isothermal amplification from sample collection, reagent preparation, amplification, and detection using various techniques. B) An example of an IAT using CRISPR-Cas technology.

Table 3
Examples of recent IAT applications in SARS-CoV-2/COVID-19 detection.

RT-IAT	Sample	Target	Signal readout	LOD (copies/μL)	Temp (°C)	Reaction time	Sensitivity	Refs
LAMP	20 NP	N	Fluorometric	50	65	~30 min	100%	[42]
RPA	133 NP	N	Colorimetric, LFIA	7.659	37	20 min	98%	[43]
TMA	116 NP	ORF1ab	Luminescence	5.5	–	2.4 h	98%	[44]
NEAR	61 NP	RdRp	Fluorometric	0.125	60	~15 min	71.7%	[45]
CRISPR-Cas 12	78 NP and OP	N, E	Colorimetric	10	37, 42	<40 min	95%	[46]
CRISPR-Cas 13	154 NP and throat	ORF1ab, S, N	LFIA, fluorometric	10	37, 42	35–70 min	96%	[47]
SDA	164 OP	RdRp, N	Fluorometric	10	42	<30 min	96.77%	[48]

SARS-CoV-2/COVID-19 – severe acute respiratory syndrome coronavirus 2/coronavirus disease 2019; RT-IAT – Reverse transcription isothermal amplification test; LOD - limit of detection; NP – nasopharyngeal; OP – oropharyngeal; LAMP – loop-mediated isothermal amplification; RPA – recombinase polymerase amplification; TMA – transcription-mediated amplification; NEAR - nicking enzyme-assisted reaction; CRISPR – clustered regularly interspaced short palindromic repeat; SDA – strand displacement and amplification.

19 diagnostics, with some even gaining US FDA-EUA to be used at POC.

3.2. Rapid SARS-CoV-2 antigen and antibody detection tests

Unlike NAATs where detection is based on nucleic acids, rapid antigen and antibody diagnostic test kits (Ag-RDT and Ab-RDT) rely on proteins and antibodies secreted by COVID-19 patients for successful detection of SARS-CoV-2. The key advantages of these two tests compared with the gold standard RT-PCR include rapidity (~15 to 30 min), ease of operation, low cost, and portability. With the ongoing pandemic, it has been suggested that these two tests could help in the mass screening of COVID-19 cases. However, they cannot be used solely for COVID-19 diagnosis owing to sensitivity issues [7,24,49]. Hence, RT-PCR should still be used to confirm results acquired by these tests. Currently, several of these test kits

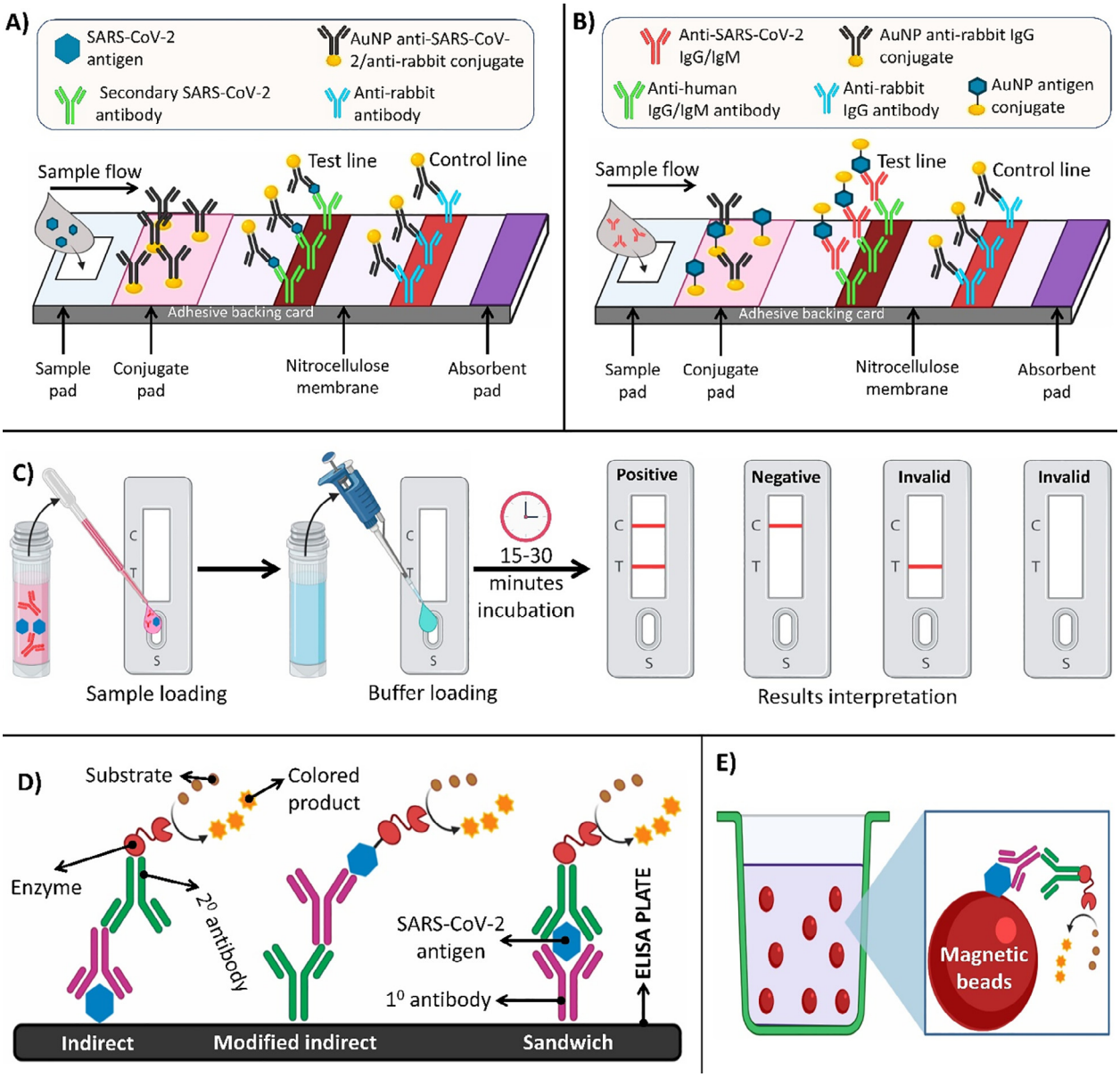

Fig. 3. SARS-CoV-2 antigen and antibody detection methods. LFIA detection of A) SARS-CoV-2 antigens, B) SARS-CoV-2 human-specific antibodies, and C) expected results interpretation. D) Commonly used ELISA techniques and E) magnetic bead-based CLIA assay for the detection of SARS-CoV-2 human antibodies.

have gained US FDA-EUA approval for the diagnosis of SARS-CoV-2, as summarized in Table 2. The majority of the approved Ag/Ab-RDTs rely on the lateral flow technique, which often gives a visible readout. Below is a brief summary of the working principles of the two RDTs and other serological tests.

3.2.1. Rapid antigen tests (Ag-RDTs)

Ag-RDTs are designed to detect the S and N proteins of SARS-CoV-2 in upper respiratory tract specimens collected from COVID-19 patients. The most commonly used Ag-RDT for SARS-CoV-2 diagnosis uses the lateral flow immunoassay (LFIA) principle, as summarized in Fig. 3A. Briefly [50], antigens in the swab sample are loaded into a well with a sample pad and flow through capillary motion up to the conjugate pad containing SARS-CoV-2 monoclonal antibodies (mAbs) and control rabbit mAbs both tagged with detector molecules, such as colloidal gold nanoparticles (AuNPs). In the event of a positive sample, SARS-CoV-2 antigens link to the bound AuNPs at the conjugation pad forming a complex that moves by capillary action to the test line containing other SARS-CoV-2 antigen-specific mAbs. Here, antigen–antibody complexes become trapped forming a calorimetrically visible line that can be seen by the naked eye or with the help of a detector indicating sample positivity. Subsequently, the buffer containing rabbit mAbs migrates further down to the control line where they are captured by specific anti-rabbit antibodies. If the test is performed accurately, the control line also becomes calorimetrically visible meaning that the test is valid [50]. If not, the test is interpreted otherwise as in Fig. 3C. Apart from LFIA, high-throughput Ag-RDTs can also be achieved using automated or semi-automated technologies including enzyme immunoassays (EIA), microfluidic immunofluorescence assay (MIA), and chemiluminescence immunoassays (CLIAs) [24,50]. The sensitivity of Ag-RDTs differs depending on the test kit. A recent *meta*-analysis reviewed several Ag-RDTs and found their sensitivity to be 76.3% when used according to the manufacturer's instructions, 88.2% using an instrument, and 74.8% without the use of an instrument. The study

Table 4
Other methods used for the detection of SARS-CoV-2-specific human antibodies.

Method	Samples	Protein target	Detection Antibody	Notes	Reaction time	Ref
Indirect (IgM) and modified indirect (IgG) ELISA	238 sera	N	IgM or IgG	Specificity was found to be 100% in healthy people and 94.3% in ordinary patients; Positivity rate of ELISA was greater than that of RT-PCR and increased with disease progression; ELISA can complement RT-PCR	~2h	[56]
Sandwich ELISA	Multiple sera	N	IgM and IgG	The sensitivity and specificity for IgM was 77.3% and 100% respectively while for IgG it was 83.3% and 95%; Antibody detection can be done in middle and later stages of disease progression; ELISA can complement RT-PCR	>2h	[57]
MCLIA (Double-sandwich immunoassay)	285 Sera	S	IgM and IgG	After 19 days of onset symptoms 100% of patients tested positive; CLIA can be used to complement RT-PCR and for rapid earl screening	Not stated	[52]
FMI	Multiple sera	S, S1, S2, and N	IgM, IgG, and IgA	Sensitivity and specificity increased from 86% and 100% in the first week to 100% in the second week after symptoms onset	>3h	[58]

SARS-CoV-2/COVID-19 – severe acute respiratory syndrome coronavirus 2/coronavirus disease 2019; MCLIA – magnetic chemiluminescence enzyme immunoassay.

also noted that the sensitivities can increase in all testing platforms to 95.8% and 83.8% when tests are performed on specimens with high viral loads or on patients after 1 week of symptoms onset, respectively [49]. Despite this increase, the sensitivity is still low compared with RT-PCR emphasizing the need to further confirm results by RT-PCR before COVID-19 diagnosis.

3.2.2. Rapid antibody tests (Ab-RDTs)

Given the disease progression of COVID-19 within 1–2 weeks, Ab-RDTs and other serological tests have limited use in the diagnosis of acute SARS-CoV-2 infection but are useful after the immune response has been elicited over time [51–53]. Similar to the antigen tests, most Ab-RDTs use LFIA with the target being specific to one or more human antibodies (i.e., IgA, IgG, and/or IgM) against SARS-CoV-2, as summarized in Fig. 3B. Common samples used in LFIA Ab-RDTs include saliva and blood/serum. Briefly, after the sample is loaded into the sample well, it migrates through capillary motion to the conjugate pad containing AuNP-tagged SARS-CoV-2 antigens and AuNP-tagged control antibodies. Here, anti-SARS-CoV-2 IgA, IgG, or IgM binds to the AuNP-tagged antigens and moves to the test line containing anti-human IgA, IgG, or IgM antibodies. If the sample is positive for SARS-CoV-2, the (IgA, IgG, or IgM)–AuNP-tagged antigen complex binds to the anti-human IgA, IgG, or IgM antibodies immobilized at the test line, resulting in a visible colorimetric line indicating a positive sample. The AuNP-tagged control moves further to the control line to form a calorimetrically visible line that indicates a valid/successful test. If the test is invalid or negative, the results can be determined as in Fig. 3C. In addition to LFIA, scientists are exploring other methods to sensitively and rapidly detect SARS-CoV-2 antibodies. For example, Elledge *et al.* used rationally designed split luciferase antibody biosensors to detect antibodies in whole blood, serum, plasma, and to a lesser extent, saliva, within 30 min [54]. Despite these advancements, a gold standard method to validate both Ag- and Ab-RDTs remains lacking. Apart from Ab-RDTs, other slower methods also exist, as summarized in Table 4 and Fig. 3D and E, for the detection of human-specific SARS-CoV-2 antibodies. These methods include enzyme linked immunosorbent assays (ELISA), CLIAs that employ indirect and sandwich ELISA techniques, and fluorescent microparticle immunoassays (FMI), all of which have been reviewed in detail elsewhere [7,24,54,55].

4. Conclusion and perspectives

The ongoing SARS-CoV-2/COVID-19 pandemic has emphasized the importance of molecular diagnostic tools to control infectious disease outbreaks. Since the beginning of the pandemic, scientists and technicians have worked on the frontline to develop diagnostic solutions for SARS-CoV-2. Among these, RT-PCR is the most commonly used technique and remains the gold standard for the detection of SARS-CoV-2. Owing to some of its limitations, including speed and portability, alternative NAATs and RDTs have been explored. These tests, including Ag/Ab-RDTs, are suitable for the surveillance of SARS-CoV-2/COVID-19 and POC testing due to increased speed, portability, affordability, and ease of operation. A major limitation of RDTs compared with NAATs is the lack of sensitivity, hence diagnostic data still need to be confirmed by RT-PCR. Ag/Ab-RDTs also lack a validation standard. More work is needed to improve the sensitivity of RDTs and to develop validation standards. The FDA is responsible for EUA of diagnostic tests that can reliably diagnose SARS-CoV-2 *in vitro*. From our findings, the majority of kits approved by this body are dependent on RT-PCR. More work is also needed on other NAATs, IATs, and RDTs to match the growing list of RT-PCR-approved FDA-EUA kits. When using these molecular diagnostic tools, laboratories should perform site-specific and activity-specific risk assessments to ensure biosafety procedures are followed in detail. Personnel should also demonstrate competency in performing the tests in strict adherence to relevant protocols at all times. This will ensure results are reported accurately, while reducing the risk of laboratory accidents including cross-contamination and cross-infection. The WHO, CDC, and other related organizations should strive to continuously update and revise the biosafety protocols and techniques related to SARS-CoV-2/COVID-19 to help laboratories across the globe combat SARS-CoV-2/COVID-19. Future pandemics are now more navigable owing to these advancements in molecular diagnostic tools and biosafety protocols.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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