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ORIGINAL ARTICLE

Progress of the Detection Methods for SARS-CoV-2

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SUMMARY

Background: **The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) causes a respiratory illness, characterized by symptoms such as fever, dry cough, and drowsiness. This virus is highly contagious, has significant mutation rates, and induces infection despite vaccination. Its widespread prevalence has profoundly impacted global economies, societies, and daily life. In response to these challenges, researchers have committed themselves to advancing rapid and cost-effective diagnostic technologies, holding substantial importance for the rapid evolution of global diagnostic capabilities. Nonetheless, various detection methods diverge in principles, sensitivity, specificity, and other aspects. Additionally, COVID-19 is not an isolated event, but part of a broader history of pandemics in human society. Therefore, this article briefly reviews the existing detection methods of SARS-CoV-2, providing valuable technical insights to diagnose not only SARS-CoV-2 but also other viruses.**
Methods: **A search was conducted on PubMed by utilizing keywords such as "SARS-CoV-2 detection", "RT-qPCR detection for SARS-CoV-2", "LFA detection for SARS-CoV-2", "Biosensors detection for SARS-CoV-2", and similar terms. The objective was to compile and summarize relevant articles on these topics.**
Results: **Currently, the real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) stands as a widely employed method for detecting SARS-CoV-2, enabling an accurate detection of viral RNA. Furthermore, the lateral flow assay (LFA) assists in detecting viral antigens and antibodies. Gene sequencing technology primarily facilitates the real-time monitoring of mutated SARS-CoV-2 strains, while biosensors could offer a rapid, economical, sensitive, and precise detection of SARS-CoV-2. These methods provide a strong technical support for the early detection and diagnosis of SARS-CoV-2.**
Conclusions: **This paper offers a concise overview of pathogen detection methods, as molecular biology, and immunological detection techniques, alongside emerging biosensor platforms relevant to SARS-CoV-2, and delineates the strengths and weaknesses of each method.**
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KEYWORDS

SARS-CoV-2 detection methods, nucleic acid-based assays, immunological tests, biosensor-based tests

LIST OF ABBREVIATIONS

4-ATP - 4-Aminothiophenol
ACE2 - Angiotensin-converting enzyme 2
AcpcPNA - Pyrrolidinyl peptide nucleic acid
AP - Auxiliary probe
APTES - (3-Aminopropyl) triethoxysilane
Atto MB2 - A methylene blue derivative
AuNPs - Gold nanoparticles

bp-SNA - Bipolar silica nanochannel array
BSA - Bovine serum albumin
BSL-3 - Biosafety level 3
CARMEN - Combinatorial arrayed reactions for multiplexed evaluation of nucleic acids
CC - Carbon cloth
cDNA - Complementary DNA
c-g-C3N4-2D - Carbonylated graphitic carbon nitride
CLIA - Chemiluminescent immunoassay
CNC - Cellulose nanocrystals
CNFs - Carbon nanofibers
CNTs - Carbon nanotubes
COVID-19 - Coronavirus disease 2019
CP - Capture probe
CRISPR - Clustered regularly interspaced short palindromic repeats
Ct - Cycle threshold
CV - Cyclic voltammetry
ddPCR - Droplet digital PCR
DNHCR - DNA nanostructure hybridization chain reaction
dPCR - Digital PCR
DPV - Differential pulse voltammetry
E - Envelope
ECL - Electrochemiluminescence
EIS - Electrochemical impedance spectroscopy
ELISA - Enzyme-linked immunosorbent assay
ERGIC - Endoplasmic reticulum-Golgi intermediate compartment
Fc-IgG - Ferrocene carboxylic acid-SARS-CoV-2 antibody
FG-CNT-FET - Multi-functionalized floating gate carbon nanotube field effect transistor
FTO - Fluorine-doped tin oxide
GCE - Glassy carbon electrode
Gly - Glycine
GNPs - Gold nanoparticles
GNRs - Gold nanorods
G-PLA - Graphene polylactic
GSH-AuNPs - Glutathione-protected gold nanoparticles
HAE - Human airway epithelial
ICTV - International Committee on Taxonomy of Viruses
ITA - Isothermal amplification technology
ITO - Indium tin oxide
LAMP - Loop-mediated isothermal amplification
LFA - Lateral flow assay
LOD - Limits of detection
LP - Label probe
LSV - Linear sweep voltammetry
M - Membrane
mAb - Monoclonal antibody
MB - Methylene blue
MCH - 6-Mercapto-1-hexanol
MGEs - Mobile genetic elements
mNGS - Metagenomic next generation sequencing
MNPs - Magnetic nanoparticles
MXenes-2D - Transition-metal carbides
N - Nucleocapsid
nAbs - Neutralizing antibodies
NPs - N-terminal peptides
NSPs - Non-structural proteins
ORFs - Open reading frames
PANI - Polymerized polyaniline
PCBGE - Printed circuit board-based gold substrate
PCR - Polymerase chain reaction
PNA - Peptide nucleic acid
pre-crRNA - Precursor CRISPR RNA
PULD - Point-of-care upconversion luminescent diagnostic instrument
PVOCs - Particular volatile organic compounds
RBD - Receptor-binding domain
RdRp - RNA-dependent RNA polymerase
rGO-Aunano - Graphene oxide decorated with gold nanoparticles
rGO - Reduced graphene oxide
Rough ER - Rough endoplasmic reticulum
RTCs - Replication transcription complexes
RT-LAMP - Reverse transcription loop-mediated isothermal amplification
RT-PCR - Real-time reverse transcription-polymerase chain reaction
RT-qPCR - Real-time reverse transcription-quantitative polymerase chain reaction
S - Spike
SARS-CoV-2 - Severe acute respiratory syndrome-coronavirus-2
SA - Streptavidin
SCX8-RGO - Sulfonated calix[8]arene functionalized graphene
SCX8-RGO-p - P-sulfonated calix[8]arene (SCX8) functionalized graphene
SHERLOCK - Specific high-sensitivity enzymatic reporter unlocking
SKI - Skim milk
Sp-aptamer - Spike protein aptamer
SPCE - Screen-printed carbon electrode
SPE - Screen-printed electrode
SPEs - Screen-printed electrodes
SPGE - Screen-printed graphene electrode
ssDNA - Single-strand DNA
STOPCovid - SHERLOCK testing in one pot covid
SWCNT-COOH - Carboxylic acid functionalized carbon nanotubes
SWV - Square wave voltammetry
Th - Thionine
TFGEs - Thin-film gold electrodes
TMPRSS2 - Transmembrane serine protease 2
UCNPs - Upconversion nanoprobes
UTR - Untranslated region
VOCs - Variants of concern
WGS - Whole-genome sequencing
WHO - World Health Organization
ZnONR - Zinc oxide nanorod

INTRODUCTION

In late 2019 and early 2020, numerous cases of pneumonia were first reported in Wuhan, leading to global attention [1]. On February 11, 2020, the World Health Organization (WHO) had formally named the pneumonia as coronavirus infectious disease 2019 (COVID-19). Simultaneously, the International Committee on Taxonomy of Viruses (ICTV) officially identified its pathogen as severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) [2].

SARS-CoV-2 belongs to the beta-coronavirus family and is an RNA virus with a genome size of ca. 29.9 kb, including 14 open reading frames (ORFs), encoding 29 proteins. ORF1abs is located in the 5'-region, encoding a variety of proteins required for viral transcription and replication, including 16 non-structural proteins (NSPs), of which nsp12 encodes the RNA dependent RNA polymerase (RdRp) [3,4]. The genes located at 3'-UTR encode four structural proteins: spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein [5]. The S protein is comprised of S1 and S2 subunits [6], and it plays a crucial role of viral cell infection. The receptor-binding domain (RBD) located on the viral S1 protein binds to the angiotensin-converting enzyme 2 (ACE2) on the surface of host cells. Simultaneously, in the presence of transmembrane serine protease 2 (TMPRSS2), the activation of the virus endocytosis starts. Subsequently the endocytosis leads to the release of viral RNA in the host cell [2,7]. Notably, as TMPRSS2 and ACE2 are co-expressed in epithelial cells of the mucous membrane of the nose, lungs, and bronchial branches [8,9], the viral RNA gains access and replicates within these cells, as illustrated in Figure 1. Replication results in the production of viral surface proteins (S, E, M, and N) [10], which undergo further processing within the endoplasmic-Golgi network. Ultimately, under the regulation of 16 NSPs (nsp1 - nsp16), virions assemble and are released into the environment, initiating infection in other susceptible target cells [11, 12].

The transmission of this virus primarily occurs through respiratory droplets and close contact. Both symptomatic and asymptomatic individuals infected with the novel coronavirus serve as the main sources of transmission. The average incubation period ranges from 1 to 14 days, with most cases exhibiting an incubation period of 3 to 7 days. A prolonged latency period poses challenges to the timely detection of COVID-19 cases. The infectiousness is the highest 2 days before symptom onset and the early stage of the disease. The main clinical symptoms after infection include fever, fatigue, diarrhea, dry cough, and shortness of breath [13-15]. In response, Yao et al. [14] conducted a detailed study to elucidate the molecular structure of SARS-CoV-2, providing insights for the design of subsequent vaccines. However, in the face of the sudden emergence of the Omicron variant of the mutated SARS-CoV-2, even vaccinated individuals may still be susceptible to the virus infection. Therefore, seeking economic, rapid, and sensitive early diagnostic methods is an effective measure to control the COVID-19 pandemic. Conventional virus detection methods historically involved virus isolation and culture techniques, real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and gene sequencing technology. Lateral flow assay (LFA) has replaced virus isolation and culture techniques. However, the RT-qPCR possesses certain limitations such as expensive instruments, complex performance, high costs, and unsuitability for remote areas. Given the escalating demand for diagnostic techniques during the epidemic, there is an urgent necessity to innovate and develop new diagnostic technologies. Therefore, the biosensor platform has gained considerable attention due to its characteristics as a miniaturized, rapid, sensitive, and cost-effective assay, offering significant advantages in point-of-care testing.

This review describes diverse SARS-CoV-2 detection methods, including viral isolation and cultivation methods, polymerase chain reaction (PCR), clustered regularly interspaced short palindromic repeats (CRISPR), gene sequencing technology, enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), LFA, biosensors, and other relevant strategies, as depicted in Figure 2. This review seeks to dissect the inherent strengths and limitations of each method. The primary objective is to contribute knowledge to a swift and effective prevention and to control strategies against SARS-CoV-2 spread and its evolving variants. Additionally, this discussion aims to give insight in detection methods of potential future viruses that might pose threats, fostering preparedness in public health management.

Viral isolation and cultivation methods

Viral isolation and cultivation methods are essential techniques in virology, but partially very time-consuming (up to 4 - 6 weeks), expensive, and in need of special safety conditions, such as biosafety level 3 (BSL-3) for isolation of SARS-CoV-2. The procedure involves patient samples, their concentration and inoculation of cultured cells such as Vero E6, Caco 2/AT, or HuH-6/AT cells [16]. The growth of viruses within the host cells can be monitored by using various methods, including observing cytopathic effects, employing immunofluorescence, or utilizing PCR. Once propagated, the viruses can be purified, further characterized, and studied for their genetic, structural, and functional properties. This information is valuable for understanding viral pathogenesis, developing diagnostic tests, and designing antiviral therapies [15]. Initially Zhu et al. [17] and Jonsdottir et al. [18] described a method to culture coronavirus from bronchoalveolar lavage fluid. However, virus isolation has drawbacks, such as a low sensitivity, lengthy processing time, and stringent cultivation conditions, making it unsuitable for a routine diagnosis of SARS-CoV-2.

Table 1. Comparison of different SARS-CoV-2 molecular detection methods.

Methods	Target analyte	Sensitivity (%)	Specificity (%)	Testing time (hours)	Advantages	Disadvantages	Commercially availability	Cost compared to RT-PCR	Reference
RT-qPCR	Nucleic acid	95 - 100	100	2 - 4	High sensitivity, high specificity	Prone to false negative results, complex equipment, professional personnel	Yes	High	[4,34]
ddPCR	Nucleic acid	93	100	4 - 6	Can accurately detect the virus in samples with low viral load	Expensive and time-consuming	Yes	High	[30,31]
RT-LAMP	Nucleic acid	75 - 100	100	1	Does not require thermal cycler	Primer designation with high requirements	Yes	Low	[36,49]
CRISPR technology	Nucleic acid	90 - 93	98 - 100	0.6 - 1	Fast response, easy to perform, could be used at airports as SHERLOCK rapid diagnostic test	Results in low accuracy, immature system	No	High	[40,42]
Sequencing technology	Nucleic acid	99	100	24	Virus mutations can be identified	High cost, complex operation, and 24 hours are required	Yes	High	[4,45]

RT-qPCR - Real-time reverse transcription-quantitative polymerase chain reaction, ddPCR - Droplet digital PCR, RT-LAMP - Reverse transcription loop-mediated isothermal amplification, CRISPR - clustered regularly interspaced short palindromic repeats, SHERLOCK - Specific high-sensitivity enzymatic reporter unlocking.

Molecular biology techniques

Molecular biology techniques are a set of experimental methods used to study and detect DNA, RNA, and proteins. Some commonly used molecular biology techniques include PCR, DNA sequencing, gel electrophoresis, western blotting, cloning, and gene expression analysis. Molecular biology techniques provide several advantages, such as a high sensitivity, low false-negative rates, and the ability to detect pathogens within a shorter window period [19]. These methods not only can identify a wide range of pathogens, with the exception of prions, but also analyze drug resistance genes and conduct pathogen homology analysis, making them valuable for pathogen detection [20]. Table 1 provides a summary outlining the advantages and disadvantages of these technologies.

Real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR is the preferred method for SARS-CoV-2 detection [21]. It facilitates real-time monitoring of amplification products by utilizing fluorescent signals to track the amplification of the target nucleic acid throughout the PCR process. This method analyzes the OFR1ab, S gene, E gene, and nsp12 gene (RdRp) of the SARS-CoV-2 [22]. The detection process, as depicted in Figure 3, involves the extraction of viral RNA from the collected samples, followed by reverse transcription into transcribed complementary DNA (cDNA). Subsequently the cDNA is amplified by PCR. Upon reaching the detection threshold, analysis of the obtained cycle threshold (Ct) value and a standard curve are conducted to achieve SARS-CoV-2 detection [23]. Wang et al., in

Table 2. Comparison of the analytical performances of the SARS-CoV-2 immunological detection methods.

Method	Target analyte	Sample type	Sensitivity (%)	Specificity (%)	Testing Time (hours)	Advantages	Disadvantages	Commercially availability	Cost compared to RT-PCR	Reference
ELISA	IgG, IgM, IgA	blood, saliva, and respiratory fluid	87 - 100	97 - 100	1 - 3	This test can detect both recent and past exposure to SARS-CoV-2	A multistep and long turn-around-time	Yes	Low	[52,54]
LFA	IgG and IgM	blood, saliva, and respiratory fluid	86 - 100	98 - 100	< 0.25	The detection time is short and no special equipment is required	Lower sensitivity than PCR for antigen	Yes	Low	[58,62]
CLIA	mAb	blood, saliva, and respiratory fluid	94 - 100	98 - 100	1 - 2	High sensitivity, automation, and good repeatability	Susceptible to reagent stability, instrument, and equipment status	Yes	Low	[60,61]

ELISA - Enzyme-linked immunosorbent assay, LFA - Lateral flow assay, CLIA - Chemiluminescent immunoassay.

Table 3. Summary of the genosensors for SARS-CoV-2 detection (none of the described assays is presently commercially available).

Target	Electrode	Modifiers	Technique	Linear Range (copies/mL)	LOD (copies/mL)	Analysis time (minutes)	Reference
N gene	PCBGE	MCH/thiolated ssDNA/Au	EIS	6×10^{14} - 90×10^{14}	3×10^{14}	5	[73]
RdRP	SPCE	rGO-Au_{nano}/PNA	SWV	3×10^{3} - 3×10^{4}	24	135	[74]
RNA	SPEs	AuNPs/ WO3/4-ATP	EIS	6×10^{4} - 1.2×10^{18}	1.8×10^{8}	5	[75]
cDNA	G-PLA	Au/thiolated capture sequence	DPV	6×10^{14} - 3×10^{16}	1.8×10^{14}	30	[76]
RdRP	GCE	ssDNA/MXene/ Pt/C	DPV	6×10^{2} - 6×10^{12}	240	30	[77]
N gene	GCE	PANI/biotin-peptide/SA/ biotin-probe	DPV	6×10^{7} - 6×10^{12}	2.1×10^{6}	60	[78]
N gene	UCNP/cDNA	Au NPs/cDNA	ECL	1.2×10^{8} - 6×10^{12}	6.9×10^{6}	20	[68]
N gene	SPE	c-g-C3N4/ GSH-AuNPs	Chrono-amperometric	6×10^{5} - 6×10^{9}	1.3×10^{6}	60	[79]
ORF1ab	SPCE	LP/Au@SCX8-RGO-TB	DPV	6×10^{4} - 6×10^{9}	200	180	[72]

4-ATP - 4-Aminothiophenol, APTES - (3-Aminopropyl) triethoxysilane, AuNPs - Gold nanoparticles, cDNA - Complementary DNA, c-g-C3N4–2D - Carbonylated graphitic carbon nitride, CV - Cyclic voltammetry, DPV - Differential pulse voltammetry, EIS - Electrochemical impedance spectroscopy, GCE - Glassy carbon electrode, G-PLA - Graphene polylactic, GSH-AuNPs - Glutathione-protected gold nanoparticles, LOD - Limits of detection, LP - Label probe, MCH - 6-Mercapto-1-hexanol, MXenes - 2D transition-metal carbides, PANI - Polymerized polyaniline, PCBGE - Printed circuit board-based gold substrate, PNA - Peptide nucleic acid, rGO - Aunano-Graphene oxide decorated with gold, SA - Streptavidin, SCX8-RGO - Sulfonated calix[8]arene (SCX8) functionalized graphene, SPCE - Screen-printed carbon electrode, SPEs - Screen-printed electrodes, ssDNA - Single-strand DNA, SWV - Square wave voltammetry, UCNPs - Upconversion nanoprobes.

Table 4. Summary of the immunosensors for SARS-CoV-2 detection (none of the described assays is presently commercially available).

Target	Electrode	Modifiers	Technique	Linear range (copies/mL)	LOD (copies/mL)	Analysis time (minutes)	Reference
Anti-S antibodies	FTO	ZnONRs/ s-protein/Gly	EIS	1.2×10^{10} - 7.3×10^{10}	1.2×10^{9}	5	[83]
Anti-S antibodies	SPE	Peptide/AuNP	DPV	4.5×10^{9} - 9.1×10^{12}	1.8×10^{9}	60	[84]
Anti-S antibodies	SPE	rGO-peptide/ BSA	DPV	4.8×10^{9} - 3.2×10^{12}	4.7×10^{10}	60	[85]
Anti-S1 antibodies	SPCE	MNPs/ S1 protein	DPV	1.5×10^{8} - 1.2×10^{10}	5.6×10^{7}	30	[86]
Anti-S antibodies	ITO	Bio-Antigen/ SA/Ru-bp-SNA	ECL	3.1×10^{5} - 6.1×10^{10}	1.8×10^{5}	30	[87]
S Protein	Pt electrode	SWCNT-COOH/ GNP-Th/S1-Antibody	SWV	3×10^{11} - 6×10^{13}	1.2×10^{11} (PBS); 3×10^{11} (sample)	5	[88]
S Protein (RBD)	SPGE	SKI/Antibody/ CNC	DPV	6×10^{3} - 5.1×10^{9}	120	120	[81]
S Protein (RBD)	SPGE	Fc-IgG/ACE2	DPV	6×10^{5} - 5.1×10^{9}	1.8×10^{5}	12	[89]
S Protein (RBD)	SPE	NPs/MB-GO	CV	6.1×10^{4} - 6.1×10^{10}	3.5×10^{4}	1	[90]
S Protein (RBD)	FTO	GNR/RBD-Antibody	DPV	6×10^{5} - 6×10^{14}	4.4×10^{5}	0.5	[91]

bp-SNA - Bipolar silica nanochannel array, BSA - Bovine serum albumin, CNC - Cellulose nanocrystals, CV - Cyclic voltammetry, DPV – Differential pulse voltammetry, ECL - Electrochemiluminescence, EIS - Electrochemical impedance spectroscopy, Fc-IgG - Ferrocene carboxylic acid-SARS-CoV-2 antibody, FTO - Fluorine-doped tin oxide, GCE - Glassy carbon electrode, GNPs - Gold nanoparticles, GNRs - Gold nanorods, ITO - Indium tin oxide, LOD - Limits of detection, NPs - N-terminal peptides, MNPs - Magnetic nanoparticles, rGO - reduced graphene oxide, SKI - Skim milk, SPCE - Screen-printed carbon electrode, SPE - Screen-printed electrode, SPGE - Screen-printed graphene electrode, SWCNT-COOH - Carboxylic acid functionalized carbon nanotubes, SWV - Square wave voltammetry, Th - Thionine.

Table 5. Summary of the aptamer sensors for SARS-CoV-2 detection (none of the assays is commercially available).

SARS-CoV-2 target	Substrate	Bioreceptor	Technique	Linear Range (copies/mL)	LOD (copies/mL)	Analysis time (minutes)	Reference
N Protein	Nano-pipettes	Aptamer-functionalized	LSV	6.1×10^{7} - 6.1×10^{11}	4.4×10^{6}	60	[94]
S Protein	FG-CNT-FET	AuNPs/Sp-aptamer	Potentio-metry	61 - 6.1×10^{5}	6.1	5	[95]
S Protein	CC	AuNPs/Thiolated aptamer	DPV	0 - 6.1×10^{10}	6.6×10^{6}	30	[96]
S Protein (RBD)	Gold electrode	aptamer-Atto MB2	SWV	4.6×10^{7} - 4.6×10^{9}	–	5	[97]
S Protein (RBD)	SPCE	AuNPs/thiolated aptamer	EIS	6×10^{9} - 1.5×10^{13}	7.8×10^{8}	40	[98]
S Protein (RBD)	SPCE	CNF-AuNP/thiolated	EIS	6×10^{9} - 3.9×10^{13}	4.2×10^{9}	40	[99]

Atto MB2 - A methylene blue derivative, CC - Carbon cloth, DPV - Differential pulse voltammetry, EIS - Electrochemical impedance spectroscopy, FG-CNT-FET - Multi-functionalized floating gate carbon nanotube field effect transistor, FTO - Fluorine-doped tin oxide, LOD - Limits of detection, LSV - Linear sweep voltammetry, Sp-aptamer - Spike protein aptamer, SPCE - Screen-printed carbon electrode, SPEs - Screen-printed electrodes, SWV - Square wave voltammetry, TFGEs - Thin-film gold electrodes.

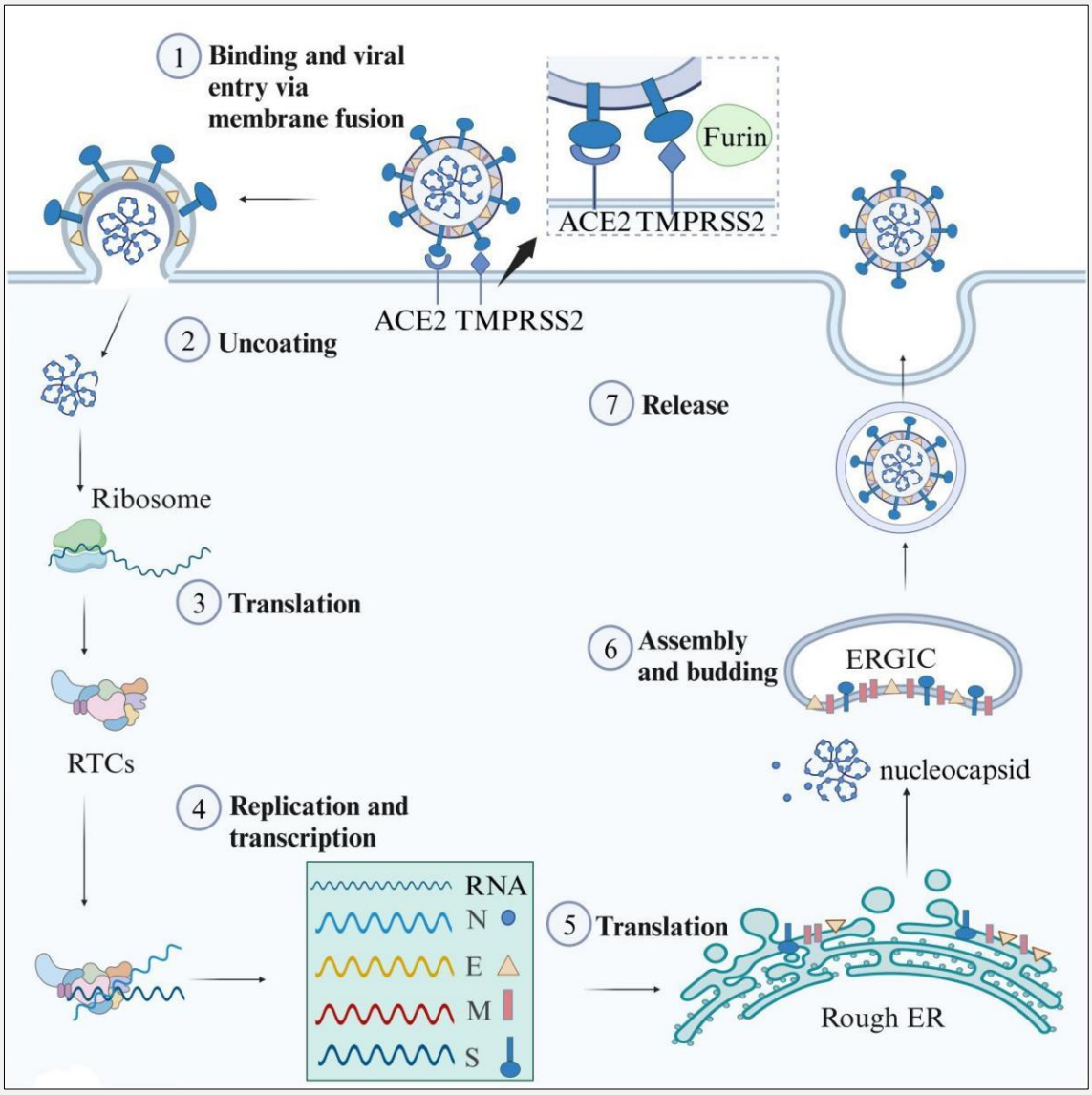

Figure 1. The life cycle of SARS-CoV-2 in host cells.

The illustration was created by using BioRender.com. ① Coronavirus particles bind to ACE2 receptors on the surface of host cells, and promote the fusion of virus and cell membrane under the combined action of TMPRSS and furin. ② Following entry, virus particles are released and genomic RNA is uncoated. ③ At ribosomes the RNA is translated in single NSPs, which are then assembled into viral RTCs. ④ The RTCs continuously replicate and transcribe a series of subgenomic RNAs. ⑤ Subgenomic RNA is translated into structural proteins S, E, M, and N in the rough ER ⑥ The viral genomic RNA and proteins are assembled and budded to form virus particles in the ERGIC. ⑦ Finally, the virus fuses with the plasma membrane of the host cell, releasing the viral particles out of the cell.
ACE2 - Angiotensin-converting enzyme 2, E - Envelope, ERGIC - Endoplasmic reticulum-Golgi intermediate compartment, M - Membrane, NSPs - Non-structural proteins, N - Nucleocapsid, Rough ER - Rough endoplasmic reticulum, RTCs - Replication transcription complexes, S - Spike, TMPRSS2 - Transmembrane serine protease 2.

2020 [24], evaluated the quality of six approved commercial nucleic acid detection kits, and the results showed that the limits of detection (LOD) of different kits varied greatly, and the worst LOD may show false negative results. Furthermore, in response to the appearance of variant strains and to enhance detection sensitivity, Tombuloglu et al. [25] have described a multiplex RT-qPCR technique to diagnose SARS-CoV-2.

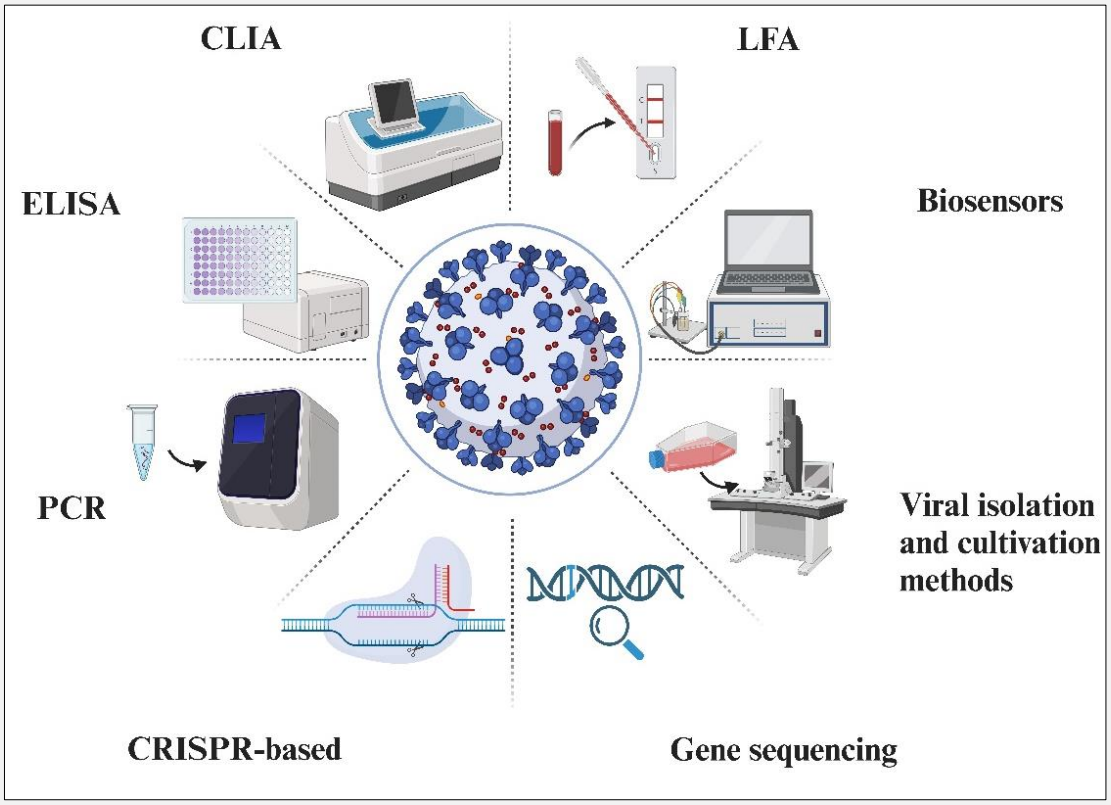

Figure 2. Methods for SARS-CoV-2.

The illustration was created by using BioRender.com. Methods for SARS-CoV-2 include viral isolation and cultivation, PCR, CRISPR-based, gene sequencing, ELISA, LFA, CLIA, and biosensors.
CRISPR - Clustered regularly interspaced short palindromic repeats, CLIA - Chemiluminescent immunoassay, ELISA - Enzyme-linked immunosorbent assay, LFA - Lateral flow assay, PCR - Polymerase chain reaction.

This assay concurrently targets two viral genes (RdRP and E) and has demonstrated 100% consistency with the detection system approved by the United States Centers for Disease Control and Prevention. In summary, RT-qPCR offers a high sensitivity, high specificity, ease of use, cost-effectiveness, and the capability to detect trace amounts of SARS-CoV-2 RNA from various sample sources [26]. Nevertheless, the accuracy of this method may be influenced by various factors, including the nature of clinical samples, timing of sampling, and the quality of reagent kits, potentially leading to false-negative results [27].

Digital PCR (dPCR)

dPCR is a nucleic acid quantification technique that allows quantification of DNA molecules. Compared to RT-qPCR, dPCR directly counts the number of DNA molecules, enabling the quantification when starting amplification. The basic principle involves partitioning the sample into thousands or millions of partitions before amplification. After PCR amplification, the fluorescence signal from each partition is detected. By applying the principles of the Poisson distribution, the initial copy number or concentration of the molecules can be accurately determined [28]. One commonly employed method for detecting SARS-CoV-2 via dPCR is the utilization of droplet digital PCR (ddPCR). Suo et al., in 2020 [29], demonstrated that ddPCR has a significantly lower LOD compared to RT-qPCR in detecting the N gene and ORF1ab gene of SARS-CoV-2. This suggests that ddPCR holds an advantage by reducing false positive results compared to RT-qPCR, potentially serving as an effective complement to the RT-qPCR methodology; whilst Falzone et al., in 2020 [30], demonstrated that ddPCR showed a higher sensitivity and specificity than RT-qPCR in the diagnosis of SARS-CoV-2 in samples with a low viral load. Additionally, Xu et al. [31] used ddPCR for clinical evaluation of patients in-

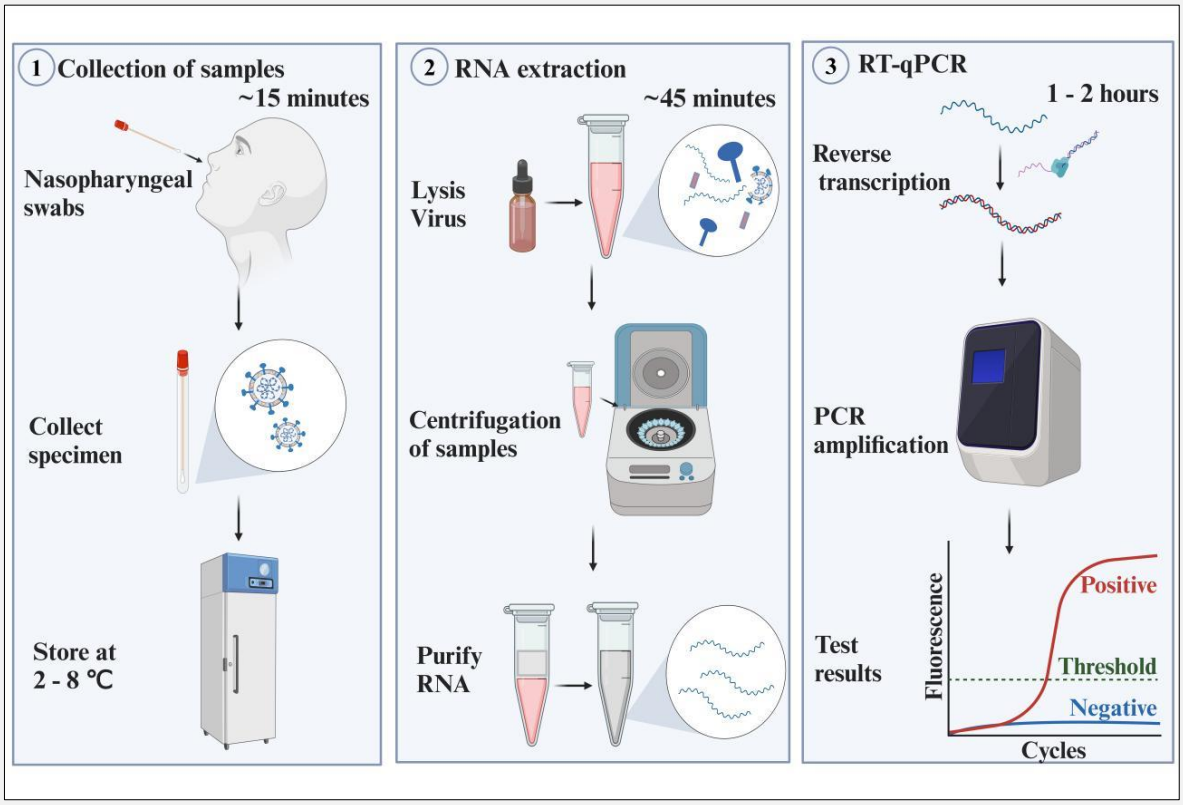

Figure 3. Performance steps of the SARS-CoV-2 RT-qPCR diagnostic test.

The illustration was created by using BioRender.com. ① Collection of nasopharyngeal swab and storage in the refrigerator at 2 - 8°C. The process takes about 15 minutes. ② Virus RNA was extracted by lysis of the virus, centrifugation, and purification of the sample. The process takes about 45 minutes. ③ RNA transcription, amplification, fluorophore hydrolysis, and readout. The process takes about 2 - 3 hours.

fected with a low amount of SARS-CoV-2, achieving a LOD of 50 copies/mL. Their study revealed a 97.9% consistency in positive results, achieving complete agreement (100%) in test negative samples, and accurately identified 9 of 10 test borderline samples. The study revealed that ddPCR offered a precise assessment of borderline positive or suspected false negative cases. However, the additional time requirement of approximately 15% and the additional costs of 5 - 10%, compared to RT-qPCR, limit its broader application.

Isothermal amplification technology (ITA)

ITA is a recent advancement method for DNA or RNA amplification performed at a constant temperature. This technology amplifies genetic material at a constant temperature through a single repeated reaction cycle, simplifying equipment and shortening reaction time. Despite this distinction, ITA achieves amplification yields comparable to traditional PCR methods [32]. Moreover, in comparison to traditional PCR, ITA typically does not necessitate a thermal cycler [33]. Depending on primer design and amplification principles, ITA can be classified into various methods such as loop-mediated isothermal amplification (LAMP), rolling circle amplification, strand displacement amplification, helicase-dependent amplification, recombinase polymerase amplification, nucleic acid sequence-based amplification, and others [32]. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is the commonly used method for SARS-CoV-2 detection. This technique employs primers designed for 6 to 8 specific regions of the SARS-CoV-2 gene. The target gene is amplified within an hour at a constant temperature of 65°C, utilizing strand displacement DNA polymerase. The test results can be observed by using colorimetric indicator methods [34]. He et al. [35] developed a single-tube colorimetric RT-LAMP technique for visually detecting SARS-CoV-2. This method integrates magnetic beads coated with silica-coated silicon hydroxyl groups for rapid RNA extraction and fast isothermal

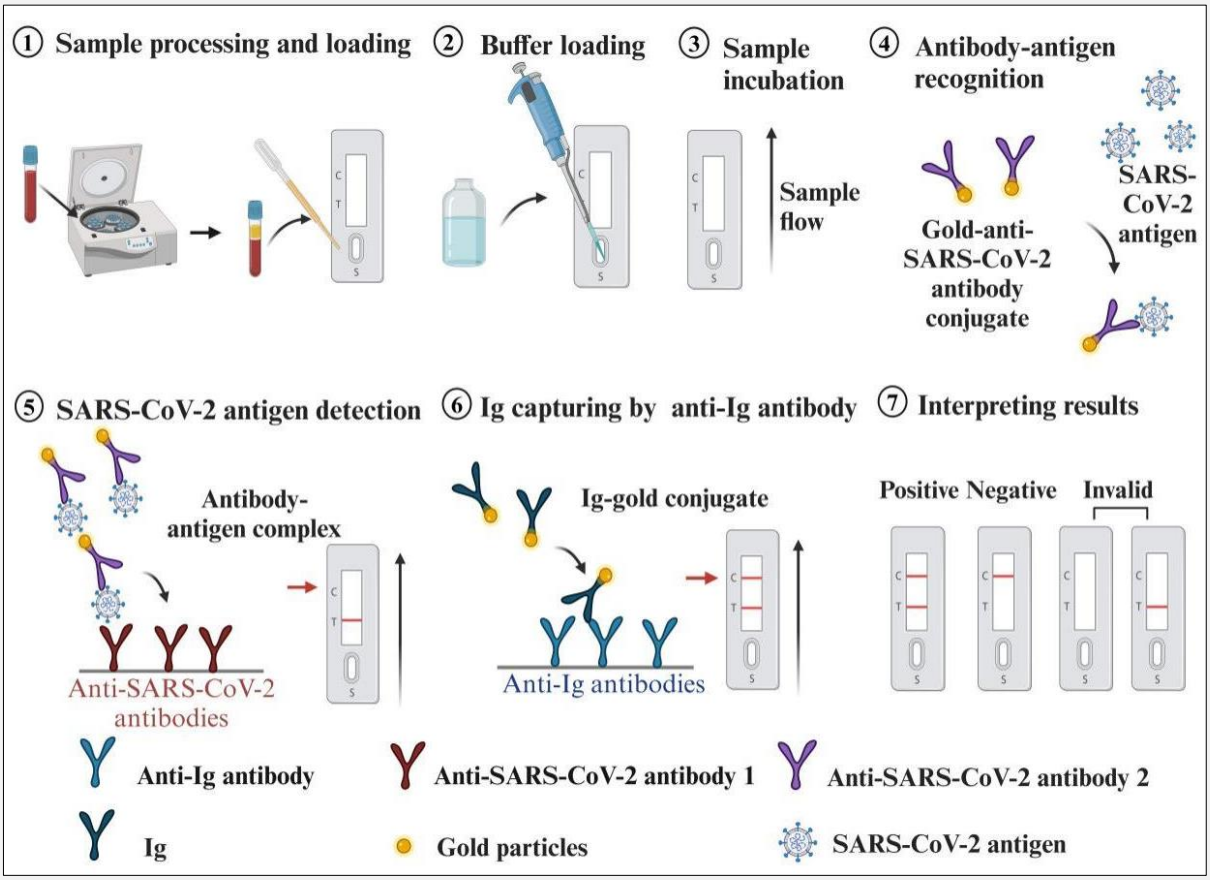

Figure 4. Analytical workflow for the rapid detection of SARS-CoV-2 viral antigens through LFA.

The illustration was created by using BioRender.com. ① Process the patient's blood sample and drop to the sample pad. ② Add a few drops saline buffer. ③ Wait for incubation. ④ Gold labeled SARS-CoV-2 antibody reaction. ⑤ Antigen-antibody complex color reaction with anti-SARS-CoV-2 antibodies on the test line. ⑥ Ig-gold conjugate color reaction at the control line. ⑦ Interpretation of the results.

amplification in a single tube, eliminating the RNA elution step by directly amplifying RNA molecules on the magnetic beads for visual result determination. Its ability to rapidly conduct reactions in a single tube makes it especially suitable for use in community clinics or township hospitals. Furthermore, studies have indicated that when RT-qPCR and RT-LAMP are employed side by side, diagnostic sensitivity can range from 75% to 100%, with a specificity of 100% [36]. Nevertheless, this method demands precise primer design and relies on the visual result observation, potentially introducing some subjectivity into the process [37].

CRISPR-based detection technology

CRISPR stands for "clustered regularly interspaced short palindromic repeats", representing a defense system that bacteria and viruses have developed during their evolutionary struggle [38]. As a response to foreign viral genes, bacteria gradually evolved the CRISPR-Cas system, a mechanism that allows bacteria and archaea to protect themselves against the invasion of mobile genetic elements (MGEs). When a phage initially invades bacteria, specific CRISPR-associated proteins (Cas enzymes) recognize and cleave these phages. The nucleic acid fragments of the cleaved invaders are then inserted into the repeat region of CRISPR loci, leading to the formation of precursor CRISPR RNA (pre-crRNA). Upon subsequent viral invasions, the transcription products of the CRISPR sequences undergo nuclease-mediated processing to generate mature crRNA. These crRNAs are capable of binding Cas proteins, enabling the recognition and precise cleavage of the target DNA sequence. The specific recognition ability of the CRISPR-Cas system for target nucleic acid sequences makes it a valuable tool for the rapid detection of SARS-CoV-2. Gootenberg et al. [39] developed a

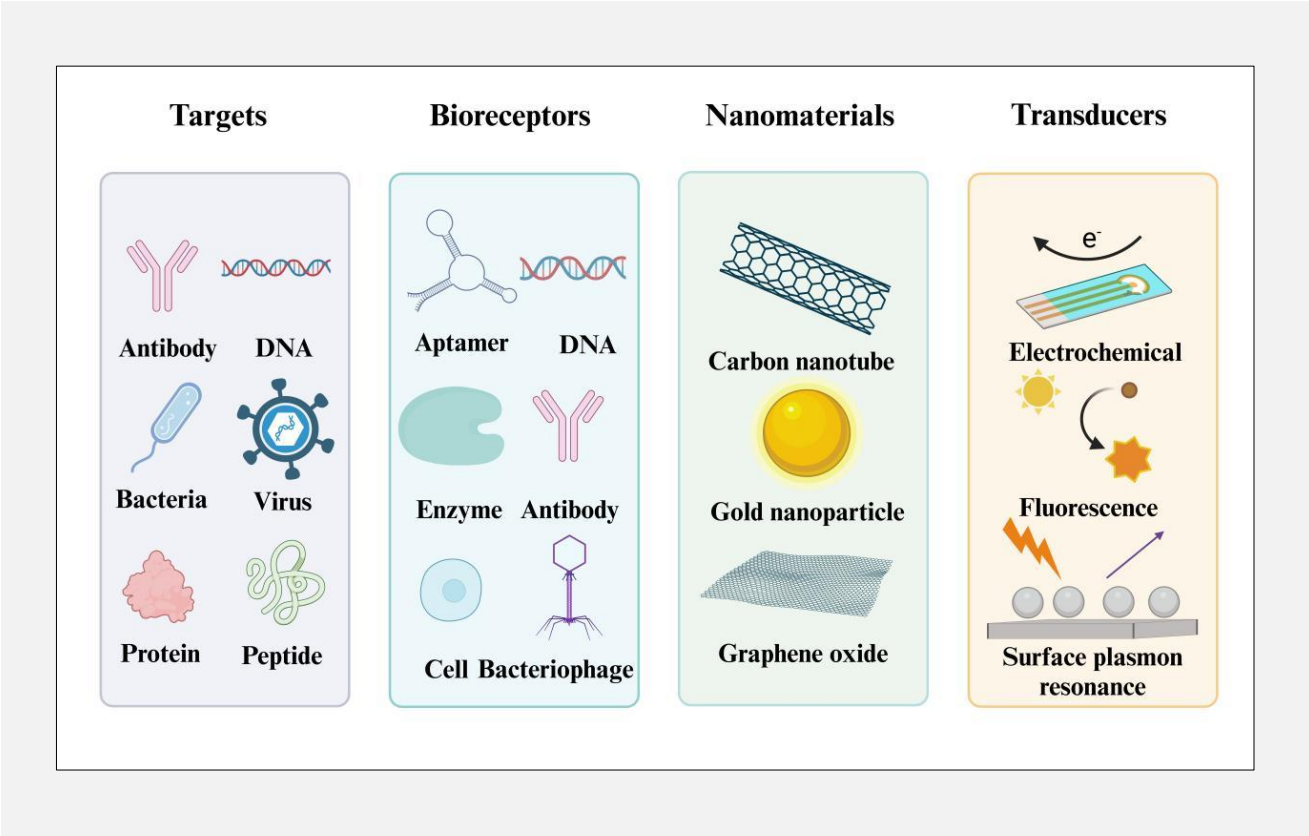

Figure 5. Components of the biosensor.

The illustration was created by using BioRender.com. Targets: Biosensors can be utilized to detect antibodies, proteins, viral and bacterial components, peptides, DNA, and other substances. Bioreceptors: Biosensors commonly employ aptamers, DNA, enzymes, antibodies, cells, bacteriophages, and other recognition elements as bioreceptors. Nanomaterials: Gold nanoparticles, carbon nanotubes, graphene, and other materials can be utilized for immobilizing targets and enhancing biosensor sensitivity. Transducers: Biosensors convert biometric events into measurable signals through various technologies, such as electrochemical, fluorescence, surface plasmon resonance, and others. Electronic system: Biosensors amplify the signal through a signal amplifier and present the signal on a display. These components work together to enable the detection and measurement of specific analytes.

platform called specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) based on CRISPR technology, which combines recombinase polymerase amplification with Cas13a for single-molecule detection of RNA or DNA. The detection platform can differentiate individual nucleotides at extremely low concentrations and can be freeze-dried for portability. However, this platform relies on RNA extraction steps and multiple liquid handling steps, making it more complex and increasing the risk of sample cross-contamination. To address this issue, Joung et al. [40] developed a simplified detection method, named SHERLOCK testing in one pot covid (STOPCovid), that aimed at facilitating the rapid diagnosis of SARS-CoV-2. Notably, STOPCovid does not require sample extraction or complex equipment, and it can achieve rapid detection with a single liquid handling step and simple visual readout. This innovative method effectively overcomes the limitations of isothermal amplification, such as false-positive results, while demonstrating sensitivity comparable to RT-qPCR. Furthermore, Joung et al. [40] used magnetic bead purification to further simplify the STOPCovid testing process and improve sensitivity. However, it should be noted that there are currently no commercially available kits, and further optimization is required. Ackerman et al. [41] developed combinatorial arrayed reactions for multiplexed evaluation of nucleic acids (CARMEN). This is a scalable platform for detecting multiple pathogens, essentially integrating a CRISPR-Cas-based nucleic acid detection system with a microwell array system. On the CARMEN platform, the detection mixture containing Cas13, sequence-specific crRNA, and a cleavage reporter gene, or amplified samples, are first individually emulsified in a microfluidic plate. They are combined with fluorescent barcodes as optical identifiers. After emulsification, all droplets from the amplified samples and detection mixture are pooled into a single tube and added to a microwell array chip. Subsequently, the two types of droplets spontaneously pair and combine within the microwells. Finally,

the test results for the crRNA of each sample are displayed through fluorescence. By combining the CARMEN platform with Cas13, reliable testing of over 4,500 crRNA target pairs on a single array can be achieved, allowing for the simultaneous testing of 400 samples for SARS-CoV-2 RNA. Furthermore, the specific recognition exhibited by Cas13-crRNA minimizes off-target effects often encountered in other nucleic acid detection methods. Additionally, the downsizing of the platform significantly reduces testing costs by approximately 300 times, but this test is not commercially available. Broughton et al. [42] introduced a CRISPR-Cas12-based detection system termed DNA endonuclease-targeted CRISPR trans reporter (DETECTR) for identifying SARS-CoV-2 infection. This method utilizes RNA obtained from nasopharyngeal or oropharyngeal swab patient samples, employing RT-LAMP for simultaneous reverse transcription and isothermal amplification. Subsequently, DETECTR detects predefined coronavirus sequences by using Cas12. Successful virus detection is confirmed upon reporting molecular cleavage. In comparison to RT-qPCR testing, DETECTR demonstrates a positive predictive value of 95% and a negative predictive value of 100% [42]. Additionally, it has advantages such as a rapid turn-around-time, strong single nucleotide target specificity, portability, and no requirement of a thermal cycler, making it suitable for rapid diagnostic method for locations like airports. Overall, CRISPR technology exhibits features like scalability, highly reusable nucleic acids, and ease of operation, enabling rapid comprehensive diagnostic testing in large sample sets. Its research framework and platforms are not yet fully developed [43].

Gene sequencing technology

Gene sequencing technology, also known as DNA sequencing, is the scientific process utilized to determine the precise sequence of nucleotides within a DNA molecule or a specific DNA region. This technology serves as the primary tool for deciphering the genetic blueprint and code of viruses [44]. It is primarily applied in research related to SARS-CoV-2 virus vaccines and viral mutation evolution. This technique is used to predict or diagnose infections by identifying selected parts of DNA or RNA sequences of pathogens. The technology is categorized into three generations, among which metagenomic next generation sequencing (mNGS) is the widely preferred and extensively used approach. Chen et al. [45] confirmed the presence of SARS-CoV-2 by using mNGS, specifically by sequencing the viral RNA extracted from bronchoalveolar lavage fluid of COVID-19 patients on the Illumina Miseq platform. Moreover, mNGS technology can provide relevant data on SARS-CoV-2 variants such as VOCs (variants of concern), genome sequences, and gene expression. However, the sensitivity of current RT-qPCR detection targeting the N, E, and ORF1a/b genes might be affected due to the tendency of viruses to mutate as well at primer or probe binding sites. Therefore, Chan et al. [46] added nsp1 as a highly expressed gene target in clinical specimens by using nanopore whole-genome sequencing (WGS). They demonstrated that RT-qPCR developed by using nsp1 exhibits a high specificity and sensitivity. The inclusion of nsp1 gene in multiple RT-PCR detection might enhance the detection rate of SARS-CoV-2. Bull et al. [47] performed high-throughput sequencing of PCR-amplified or hybrid capture-transcribed SARS-CoV-2 by using WGS. The method presents advantages in terms of portability and affordability, and it remains uninfluenced by DNA polymerase activity. However, considering its inherent measurement bias, Mostafa et al. [48] employed mNGS for SARS-CoV-2 detection, utilizing the CosmosID bioinformatics platform to analyze SARS-CoV-2 genomic sequences. This method does not require target enrichment or amplification and can also identify potential co-infections and other microorganisms that may affect patient prognosis. Nevertheless, it is time-consuming, expensive, and requires skilled operators, which hinders its widespread application.

Immunological techniques

After the invasion of a virus into the human body, the immune system is activated, resulting in the release of specific antibodies tailored to combat the invading pathogen. Immunological techniques utilize the principle of antigen-antibody specific binding to detect antigens and/or antibodies in bodily fluids. Unlike molecular biology techniques, immunological detection methods might not offer real-time virus detection within the body. Nevertheless, they can analyze the presence of antigens and antibodies following an infection, thus also serving as an auxiliary detection technique for the novel coronavirus [50]. The commonly used clinical methods include ELISA, LFA, and CLIA. Table 2 displays the comparative analysis, outlining the similarities and discrepancies among the primary immunological detection methodologies.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA is a widely used laboratory technique to detect and measure substances such as antigens of proteins, antibodies, and hormones. ELISA involves using specific antibodies and enzymes to identify and semi-quantify the presence of a target substance in a sample [51]. This technique can effectively detect IgA, IgM, and IgG antibodies against SARS-CoV-2 RBD [52]. In comparison to highly sensitive IgA antibodies in the respiratory tract, IgG antibodies are more preferred for serum analysis due to their longer persistence [53]. Krähling et al. [54] utilized the SARS-CoV-2 S1 protein as antigen and employed enzyme-labeled anti-human IgG as the secondary antibody to design an indirect ELISA, which demonstrated a high sensitivity and specificity with still some unspecificity. The ELISA was valuable to analyze the antibody response to SARS-CoV-2 vaccines and the immunogenicity evaluation of candidate vaccines. MacMullan et al. [55] developed an

ELISA capable to detect IgG antibodies against SARS-CoV-2 in saliva samples.

Lateral flow assay (LFA)

LFA is a rapid qualitative test to detect antigens or antibodies. It operates on the basis of antigen-antibody interactions. In this assay (Figure 4), colloidal gold particles are coupled to antibodies or antigens that specifically bind to the target substance. When the sample, containing the substance of interest, is applied to the test strip, it moves along the strip via capillary forces. If the target substance is present in the sample, it attaches to the labeled colloidal gold particles. This complex is creeping along the strip membrane and is captured by specific immobilized antibodies or antigens at a test line consisting of specific antigens for antibody detection, and a control band capturing Ig, resulting in a visible signal; indicates a positive result when both lines are visible. This assay is known for its simplicity, rapidity, and user-friendliness, making it highly valuable for point-of-care testing and swift diagnostics in various settings such as healthcare facilities and field applications. It is employed for qualitative detection purposes, serving as a valuable tool for rapid diagnostics [56]. Li et al. [57] developed a test strip targeting the S1 protein, where a monoclonal antibody (mAb) against SARS-CoV-2 was conjugated with colloidal gold and immobilized. Specific monoclonal antibodies against SARS-CoV-2 and Staphylococcal Protein A were fixed on a nitrocellulose membrane as the test line and control line, respectively. Although this method is less accurate than CLIA, it has the advantage of a short processing time of 10 - 20 min. Furthermore, Li et al. [58] designed a method for detecting antibodies against the anti-S protein, which demonstrated good correlation with neutralizing antibodies (nAbs) detected by chemiluminescent microsphere immunoassay. This correlation indicates that the detection of anti-S protein antibodies through LFA can serve as an inexpensive screening to analyze protective nAbs in COVID-19 patients.

Chemiluminescent immunoassay (CLIA)

CLIA is an analytical method that combines chemiluminescence technology with antigen-antibody reactions [59]. It operates by utilizing the light emitted from a chemiluminescent compound that is linked to an antibody or antigen when an immune reaction occurs. The emitted light intensity correlates with the concentration of the target molecule, allowing semi-quantification. CLIA is highly sensitive, has a wide dynamic range, and is widely used in clinical infectious disease diagnostics and drug concentration testing. This method can be categorized into chemiluminescent labeled immunoassay and chemiluminescent enzyme immunoassay, where the former directly labels the antigen-antibody complex with a luminescent substance, while the latter employs enzyme-labeled antibodies. Xu et al. [60] developed a chemiluminescent enzyme immunoassay based on magnetic particles to detect SARS-CoV-2 N protein by coupling magnetic beads with monoclonal antibodies (mAb) against the N protein to facilitate the removal of unbound components. Subsequently, alkaline phosphatase (AP) labeled antibodies catalyzed substrate luminescence, and the concentration of SARS-CoV-2 N protein was determined based on relative luminescent units. This method exhibits an improved sensitivity and a wide linear detection range. It holds promise for a rapid diagnosis of patients with high viral loads. Furthermore, He et al. [61] demonstrated that CLIA has a high detection rate and short testing time for detecting SARS-CoV-2 IgG, showing significant advantages in terms of sensitivity. It is worth noting that IgM and IgG antibodies may have different seroconversion times and positivity rates, which can complement each other in assessing the disease progression. Therefore, in the auxiliary diagnosis of COVID-19 testing, the combined detection of IgM and IgG antibodies is often performed to improve the detection rate [61].

Detection technology based on biosensors

Biosensor is a device that can generate proportional signals based on the concentration of analytes in a reaction, allowing the measurement of biological or chemical substances. Typical biosensors consist of the following components (Figure 5): (1) biorecognition elements: biologically active substances with specific recognition capabilities for analyzing target substances; (2) transducers: turn biometric events into measurable signals, such as oxygen electrodes, photomultiplier tubes, field-effect transistors, and piezoelectric crystals [63]; (3) electronic system: used to process and convert signals, amplifying analog signals, and converting them into digital form; and (4) display: presents the results of the biosensor [64].

Biosensors, known for their rapidity, portability, high sensitivity, high specificity, and low cost, have been widely applied in fields such as life sciences [65], water pollutants [66], pharmaceutical analysis [67], and viral detection [68]. Currently, biosensors designed for SARS-CoV-2 detection can be classified into genosensors, immunosensors, and aptamer sensors (Figure 5). The sensitivity and stability of biosensors used for virus detection are enhanced by combining the recognition element with nanomaterials. Among various nanomaterials, gold nanoparticles (AuNPs) are widely used because of their biocompatibility, high electrical conductivity, and large specific surface area [69]. In addition to AuNPs, carbon nanotubes (CNTs), graphene, and other materials can also be used to immobilize the target and provide biosensor sensitivity.

Genosensors

Genosensors are specialized biosensors designed for detecting genetic material like DNA or RNA, operating on the principle of hybridization, where probes selectively bind to target sequences. Genosensors generate measurable signals, typically electrical or optical, that indicate the presence and structure of nucleic acids. Due to their

specificity and sensitivity, genosensors can be used in medical diagnostics and genetic research by identifying genetic information, mutations, pathogens, or specific DNA sequences within samples, which usually requires special instruments for electrochemical and fluorescence signal measurement and their evaluation. Jiao et al. [70] devised a rapid detection method based on DNA nanostructure hybridization chain reaction (DNHCR) for detecting SARS-CoV-2 RNA. Initially, a DNA nanostructure was constructed through self-assembly of long DNA chains and a self-quenching probe (H1). Subsequently, SARS-CoV-2 RNA initiated the hybridization of H1 and free H2 DNA probes along the nanostructure, resulting in the generation of a luminescent DNA nanostring, confirming the presence of SARS-CoV-2. The precise design of the H1 probe, coupled with the tolerance of isothermal amplification, reacts within 10 minutes at a temperature of 15 - 35°C. In comparison to traditional methods, the DNHCR-based detection method simplifies the operational steps and reduces the testing cost, thereby offering a simple and rapid alternative for conventional SARS-CoV-2 detection [71].

Additionally, Zhao et al. [72] developed an ultra-sensitive and super-sandwich-type electrochemical biosensor for SARS-CoV-2 RNA detection by using p-sulfonated calix[8]arene functionalized graphene (SCX8-RGO). The sensor employed three probe sequences: capture probe (CP), label probe (LP), and auxiliary probe (AP). CP-Au@Fe_3O_4 nanocomposites and Au@SCX8-RGO-TB- LP-AP nanocomposites were prepared by using the three probe sequences for target sequence detection. Furthermore, by employing a portable electrochemical smartphone device, this technology has been capable of detecting SARS-CoV-2 RNA without the need of nucleic acid amplification or reverse transcription. It represents a plug-and-play diagnostic system with broad prospects for rapid detection of SARS-CoV-2. Additionally, Song et al. [68] developed a smartphone-based point-of-care upconversion luminescent diagnostic instrument (PULD) for rapid detection of SARS-CoV-2 N gene. The platform utilized a distinctive current-frequency conversion-based signal detection method. By employing complementary oligo-modified upconversion nanoprobes (UCNPs) and AuNPs specifically hybridized with the target N gene, detection was achieved by quenching the fluorescence intensity induced by the luminescence resonance energy transfer effect. The test results demonstrated full consistency with RT-qPCR detection. Importantly, PULD shows a high sensitivity and specificity. Thus, this smartphone-controlled diagnostic platform enables an on-site, rapid, and ultra-sensitive detection of SARS-CoV-2 with a simple assay workflow, making PULD a promising portable tool for rapid and direct screening of various infectious diseases. The recent genosensors for SARS-CoV-2 detection are shown in Table 3.

Immunosensors

Immunosensors, a specialized type of biosensors, efficiently detect specific molecules in biological samples. They utilize immune recognition elements like antibodies or antigens, which selectively interact with the target molecule. This interaction generates a signal, commonly electrical or optical, enabling identification and quantification of the target molecule. Immunosensors play a role in medical diagnostics and research due to their rapid and precise detection of specific biomolecules within samples. Zaccariotto et al. [80] developed a novel electrochemical impedance immune sensor for SARS-CoV-2 determination based on reduced graphene oxide (rGO). This method effectively immobilizes antibodies on the large surface area of rGO and combines the high sensitivity of immune sensors to accurately detect SARS-CoV-2 S protein (RBD) in saliva samples. Compared to RT-qPCR, this sensor exhibits an enhanced sensitivity, shorter reaction time, and a simple construction process. However, the developed methods lack refined experimental investigations [80]. Jaewjaroenwattana et al. [81] successfully developed a new electrochemical paper-based antigen sensing platform for SARS-CoV-2 detection. This platform is characterized by innovative plant-based monoclonal antibodies, offering a notable cost-effectiveness. Additionally, the presence of carboxyl functional groups on cellulose nanocrystals (CNC) enhances the antibody immobilization capability, thereby improving the specificity and sensitivity. Seo et al. [82] devised a field-effect transistor (FET)-based biosensor for a rapid and highly sensitive detection of SARS-CoV-2 antigen. This sensor couples antibodies against the SARS-CoV-2 S protein to a graphene sheet, creating a FET device with the graphene sheet as the sensing region. The presence of SARS-CoV-2 leads to antibody binding and alteration of the electrical signal. This platform offers significant advantages, including a high sensitivity, label-free detection, and the absence of specific sample preparation requirements. Current immunosensors capable of SARS-CoV-2 detection are summarized in Table 4.

Aptamer sensors

Aptamer sensors are innovative biosensors that utilize aptamers, short, single-stranded DNA or RNA molecules recognized for their high specificity in binding to target molecules, such as proteins, small molecules, or even whole cells. These sensors exploit the binding affinity between aptamers and their specific targets to detect and quantify substances with remarkable precision. Aptamer sensors have gained attention for their versatility, rapid response, and sensitivity, holding immense promise in various fields including medical diagnostics and biotechnology applications. Their adaptability and high selectivity make them valuable tools for detecting and analyzing diverse biomolecules across different sample types. Given that an infection by SARS-CoV-2 prompts the body to release volatile organic compounds (PVOCs) [92], the presence of these PVOCs in exhaled

breath can serve as an indicator for screening, potentially becoming detectable during the initial stages of the infection [93]. Shan et al. [93] devised a non-invasive sensor array which relies on multiplexed nanomaterials, enabling the detection and monitoring of specific PVOCs present in exhaled breath. Additionally, the sensor array demonstrates the capability to differentiate materials of COVID-19 patients from those with other types of lung infections. The aptamer sensor array, which functions as a respiratory device, consists of eight kinds of gold nanoparticles working on a substrate, where each kind of gold nanoparticle is connected to an organic ligand, forming distinct sensing layers. When exposed to PVOCs, the sensing layers expand or contract, resulting in changes in the electrical resistance. The presence of SARS-CoV-2 specific PVOCs in exhaled breath is reflected by changes in the electrical signals. This method presents advantages such as a short collection time and ease of operation. Aptamer based tests are suitable for the rapid screening of an affected population within a limited timeframe. Table 5 provides a summary of the aptamer sensors designed for the detection of SARS-CoV-2.

CONCLUSION

This review provides an overview of the SARS-CoV-2 testing methods with the aim to provide technical support for the timely detection. RT-qPCR remains the primary diagnostic method, facilitating mass screening and diagnosis. While RT-LAMP and ddPCR face challenges to some errors and costs, genome sequencing remains vital for monitoring variants, especially with the emergence of VOCs. Tracing of infection through genome sequencing is only possible when the genome of the spreading virus is known. Immunological tests are widely used due to their technical simplicity. LFA provides simplicity and rapidity, enabling also self-testing (or home testing). Emerging technologies, like biosensors, display promise, offering speed, accuracy, and portability. These technical advancements facilitate an early detection of SARS-CoV-2. Nonetheless, further optimization is crucial for their widespread adoption. In conclusion, the rapid and accurate diagnosis plays part in controlling the epidemic. The continuous advancement in testing technologies promises an improved detection of COVID-19 patients.

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