A Review on Innovative Diagnostic Techniques for Viral Diseases

Dr. Jaya Bharti[1], Suresh Kumar Mahaseth[2], Jesbin Johnson[3]
[1]*School of Health and Allied Sciences, G D Goenka University, Gurugram, Haryana, India*
[2]*Department of Medical Laboratory Technology, DSEU Dwarka*
[3]*School of Health and Allied Sciences, G D Goenka University, Gurugram, Haryana, India*

***Abstract*— Involvement of specific and accurate diagnostic techniques is a key feature to identify and control the spread of emerging and re-emerging viral infection. However, continue improvement in diagnostics techniques are required to capture the new, emergent, and highly divergent viruses. The new pandemic of SARS CoV 2 has increased the efforts of clinical laboratories to speedily develop highly reliable diagnostic assay in order to effectively and accurately diagnose this infection, therefore limiting the spread of infection. While the structural and molecular characteristics of the SARS CoV 2 were primarily unknown but various diagnostic strategies make it possible to make a correct diagnosis of COVID 19 which are developed by private research laboratories and biomedical companies. The various immune based assay and Polymerase Chain Reaction (PCR) are the basic techniques that give the platform for advance and proficient diagnostics techniques such as real-time RT-PCR, loop-mediated isothermal amplification (LAMP), polymerase spiral reaction (PSR), biosensors, microarrays and next generation sequencing.**

Index Terms: **Diagnostic technique, re-emerging, COVID 19, RT-PCR PCR, LAMP, microarrays.**

INTRODUCTION

Global pandemic infection caused by different viruses appeared as important portion of public health concern with thousands of death annually. Whereas well-known and categorised viruses such as the human immune deficiency virus (HIV) and Hepatitis are still killing millions of people, the emerging viruses are also problematic and have caused several serious outbreaks in the recent years. For example, COVID -19, the Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) in 2002–2003, Swine Influenza A (H1N1) in 2009 and Ebola Haemorrhagic fever outbreak in 2014, accountable for thousands of deaths in Africa.

Thirty-five million HIV infected people were reported in 2013 and 350–400 million chronic carriers are find for Hepatitis B virus. As per the World Health Organization (WHO) report of 2014 (WHO, 2014); approximately 780000 people die every year due to Hepatitis B and up to 500000 death reported with Hepatitis C related hepatic diseases. Due to significantly high Morbidity and mortality rates of these diseases, efforts have been constantly done to improve clinical diagnostics.1

Recently the COVID-19 pandemic, caused by the SARS-CoV-2 virus, has affected 177,108,695 individuals, with over 3,840,223 deaths globally, as of June 18, 2021 (WHO). Due to this pandemic, living and working conditions of billions of individuals all over the world have been significantly disrupted due to mandatory improvement of social isolation and lockdowns in many cities. The world economy has significantly declined.2

Due to significantly high Morbidity and mortality rates of well establish viral diseases and new emerging viral infections have elevated the efforts for improving clinical diagnostics.

Last few years have been devoted to revolutionise the process of development of viral diagnostic techniques for treatment and management of patients with viral diseases. These techniques play vital role in the successful timely diagnosis and prognosis of the viral diseases. This has been driven by a number of factors:

1. The advances of molecular technologies and ways to make these reachable for investigative laboratories;

2. The excess of new antiviral agents and additional sophisticated understanding of how these should be used.
3. Increase sensitivity of the diagnostic value provide quick knowledge for viral loads, sequence of viral data, and antiviral resistance information.

With rapid advancements in the field of medical science and technology, the health care diagnostics industry is continuously evolving and delivering up-to-date techniques but there are several limitations which limits their application in actual diagnostic setting especially in low income group companies. This review article will focus on advance testing methods for well-established viruses with addition of new techniques for COVID 19 diagnosis, how their specific characteristics have transformed the field of diagnostic techniques and what can be done to overcome their limitations.

COMMON PRINCIPLES FOR PERFORMING LABORATORY VIRUS ASSAY

1.The proper supervision of the infectious disease is based on depends on knowledge that comes from the diagnostic methods. Specific management does not always include, or stop at, chemotherapy. Demanding and precise understanding of laboratory results guarantees real clinical management of a disease and control of its transmission.4

The various laboratory parameters affect the accuracy, Precision, sensitivity and specificity of laboratory result. The needfulness and consistency of laboratory result based on the performance and operative parameters of the planned test5. Whereas these all parameters are gives statistical values therefore have diverse clarifications and involve association with the reference method or 'gold standard' for thedesiredtest.6

Accuracy can be defined as how close the found results are to those got with the reference method and it is spoken as a ratio of correct results.

Precision describe as the reliable duplicate of one test with the same sample, and finding similar results. Internal quality control (QC) and quality assurance (QA) measures in order to preserve reliability of the test by regularly monitored these two factor. An ideal test would have 100% accuracy and 100% precision with appropriate conditions whereas external factors and working differences can cause small differences.

The parameter, Sensitivity (also called the true positive rate) is the percentage of patients with confirmed infection (by the 'gold standard' method) with positive results. It is usually measured by the lower limit of detection of the method producing a positive result.

Another parameter is Specificity, also known as negative rate. This is a non-quantitative estimate, screening the potential of the test to differentiate target from non-target sample. This measure is spoken as the percentage of infection-free patients with negative result. The nearer the result are to the reference, the higher the sensitivity and specificity of the test.

On the dissimilar, operational factor concern easiness and simple in performing the test such as the turnaround time (TAT).Which is a key routine indicator defined as the intermission time between sample registrations to result reporting. All the pre analytical steps are included in this interval. Procedure finishing point in less than one hour is perfect for completion of manufacturers aim to construct diagnosis instruments allowing shorter TAT, which is mainly use full for point-of-care settings.7

ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment free and Deliverable to end users) recognized by WHO for diagnostics in resources-limited point-of care settings 8-9

The purpose of these parameters are to deliver better management of the disease, like getting rapid result and recording of disease status , to improve clinical decision-making.

Traditional laboratory techniques for the identification of viral infections:

For diagnosis of viruses lots of traditional methods are available, from long time like

(1) Direct detection of viral antigen or viral nucleic acid in various patient material

(2) Isolation and identification of virus in cell culture and embrocated egg

(3) Serological techniques for finding and quantitative measurement of antibodies titer in the patient's serum (serology). In past few years due to advances in traditional method these direct detection

methods become capable to providing a definitive result in less than 24 hours, while virus serological assay now be converted into partial to definite purposes only.

For long time, electron microscope (EM) has been considered an efficient device for direct recognition of viruses in developed countries, through imagining and counting of the virion particles in different body fluids and histological samples. The identification is based on morphological features specific to each virus family and requires a certain amount of viral particles (up to 106 particles/ml). However, specimen preparation that must be performed beforehand may reduce the virus concentration which makes the analysis harder.10

The use of Electron Microscope with culture-based techniques has revealed great advantages in the analysis and identification of viral infections, along with serological diagnostic techniques for recognition of specific antibodies against the virus. These conventional methods are still fundamental practices in many hospital laboratories.

A summarize advantage and disadvantage of the some alternative methods to the analysis of viral infections is given in Table 1.11

Table 1- Advantages and Disadvantages of Various Virus Diagnostic Technique

S.N.	DIGNOSTIC ASSAY	ADVANTAGE	DISADVANTAGE
1	Virus isolation	• Produces further material for study of agent. • Usually highly sensitive • "Open-minded"	• Slow, time-consuming, can be difficult and expensive • Selection of cell type, etc., may be critical • Useless for non-viable virus
2.	Direct observation by electron microscopy	• Rapid • Detects viruses that cannot be grown in culture • Detects non-viable virus	• Relatively insensitive • Cumbersome for large numbers of sample • Limited to a few infections
3.	Serological identification of virus or antigen, for example, EIA	• Rapid and sensitive • Provides information on serotypes • Readily available, often as diagnostic kits	• Not applicable to all viruses • Interpretation may be difficult • Not as sensitive as PCR
4	Detection of viral genomes by PCR	• Rapid, very sensitive • Potentially applicable to all viruses incl. non-cultivable\ • Reagents (primers) for additional viruses easily made • Good quantitation of load	• High sensitivity may lead to detection of non-relevant co-infection • Risk of DNA contamination • Needs good quality control • Targeted to a specific agent
5	Complement Fixation	• Easy to perform • Convenient and inexpensive	• labour intensive and lacks sensitivity.
8	Haemagglutination inhibition	• Detection of arboviruses, influenza and parainfluenza virus subtypes and provides relative quantitation of the virus particles	• Time consuming, and demanding
6	IgM serology	• Rapid	• False positive result may occur

RECENT ADVANCE TECHNIQUES FOR THE DIAGNOSIS OF VIRAL INFECTIONS

In this review we will focus on some advance techniques which are most commonly used now a days for identification of well-established virus and new emerging viruses .Especially we are focus on advance techniques for recent pandemic, COVID 19, caused by the SARS-CoV-2 virus, which led to 177,108,695 confirmed cases, with over 3,840,223 deaths globally, as of June 18, 2021. (WHO)

One of the many challenges for containing the spread of COVID-19 is the ability to identify asymptomatic cases that result in spreading of the virus to close contacts. A study of the passengers on a Diamond Princess Cruise ship forced into temporary quarantine from an early outbreak of COVID-19, estimated the

asymptomatic proportion (among all infected cases) at 17.9% (95%Cr I:15.5−20.2%).[12]

1. Amplification-based assays: Polymerase chain reaction (PCR)

This technique invented by Mullis and Faloona in 1987 and revolutionized the field of molecular diagnosis. The basic PCR assay trusts on extraction and purification of the nucleic acid, then exponential replication of the target sequence, using a thermo stable polymerase enzyme and known primers. The resulting amplicons are then identified using a fluorescence based detection system, and the result is reported in international units IU/ml.

a) Nucleic acid amplification tests (NAAT)

After development of PCR techniques new variants of this techniques develop with modification. Nucleic acid amplification techniques (NAAT) was useful to identification of new viral variants. This techniques is well-known in the identification and management of viral infections (HBV, HCV, HIV, Influenza viruses,) because they allow determination of the viral load. In other language, quantitation of the viral nucleic acid by amplifying the target sequence thousands-fold. In most cases, they are now considered a reference, or 'gold standard' method for analytic observation such as screening of blood donor for transfusion-transmitted viruses (CMV, HIV, HCV,).[13]

b) Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

RT-PCR is based on it's amplify principle in which a small amount of viral genome in a sample is used. This technique reflected to be the gold standard for diagnosis of SARS-CoV-2 virus. Presently, COVID-19 RT-PCR test usually utilise swab sample collected from the upper respiratory tract. In some of the studies other sample like serum, stool, or ocular secretions are also used.[14-16]

recently, an RT-PCR test (TaqPath COVID-19 Combo kit) developed by Rutgers Clinical Genomics Laboratory ,uses own saliva samples, which is faster and less painful than any other specimen collection techniques. This technique can reduce the hazards to healthcare workers, and may increase number of testing.[17,18]As RT-PCR starts with laboratory change of viral RNA genome into DNA by RNA-dependent DNA polymerase (reverse transcriptase).

This reaction based on minor DNA sequence primers development to specifically identify complementary sequences on the viral RNA genome and the enzyme reverse transcriptase to construct a short complementary DNA copy (cDNA) of the viral RNA genome. In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses. This is done using a fluorescent colour dye or a sequence-specific DNA probe labelled with a fluorescent substance, as in the case of TaqMan test.

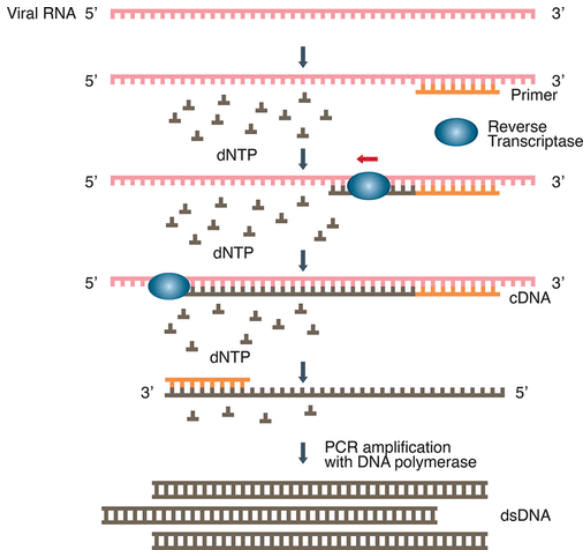

Fig 1 : RT-PCR

An automated system then repeats the replication process for about 40 cycles till the viral cDNA can be spotted, generally by a fluorescent or electrical signal.[19] RT-PCR has conventionally been carried out as a one-step or a two-step method. One-step real-time RT-PCR uses only tube containing the required primers to precede the whole reaction. Whereas in Two-step real-time RT-PCR include more than one tube to carried out the separate reverse transcription and amplification reactions, but with higher sensitivity and flexcibility. In this assay initially required small amount of material and allows for the facility to stock quantification of cDNA for several targets.[20] Normally the one-step process is the perfect method for finding of SARS-CoV-2 because it is quick to set up and involve restricted sample treatment and decrease work time and failing pipetting errors and cross-contamination among the RT and real-time PCR process. Presentably, the molecular diagnostic tests have commonly utilized the real-time RT-PCR technology target SARS-CoV-

2 genomic regions, including the ORF1b or ORF8 regions, and the nucleocapsid (N), spike (S) protein, RNA-dependent RNA polymerase (RdRP), or envelope (E) genes .[21]

The initial COVID-19 RT-PCR investigative tests included

(1) COVID-19 RT-PCR (LabCorp),[22]

(2) 2019-Novel Coronavirus Real-Time RT-PCR Diagnostic Panel [U.S. Centers for Disease Control and Prevention (CDC)],[23]

(3) TaqPath COVID-19 Combo kit (ThermoFisher Applied Biosystems), [24]

(4)Allplex2019-nCoVAssay(See gene),[25]a

(5) cobas SARS-CoV-2 (Roche).[26]

Table 2 Summary of the characteristics of common nucleic acid detection-based technologies.[27]

RT-PCR	2–3 h	Wide application range; high sensitivity; strong specificity	False-negative
Digital-PCR	3–4 h	High accuracy, high sensitivity; stable system; excellent repeatability	High cost; complicated operation; limited detection throughput; susceptible to false positives due to external contamination
Mngs	0.5–3 date	It is of great significance for the sequencing and genetic analysis of new pathogens in the respiratory tract	Short read length; uneven genome coverage; vulnerable to host genome contamination; higher cost
RT-LAMP	0.5–1 h	Easy to operate; simple and fast to operate; high sensitivity and specificity;	Vulnerable to contamination to produce false positives; low viral load to produce false negatives
CRISPR-based assays	30–40min	High specificity, high precision, high efficiency, simple and quick operation	The supporting closed detection system has not been established, which limits its wide application

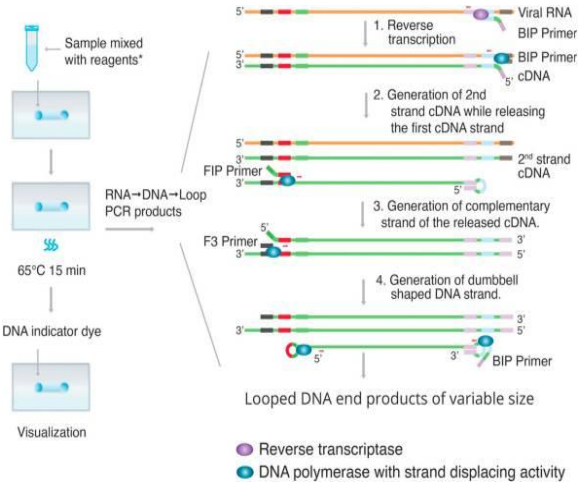

FIG:2: Reverse transcription loop-mediated isothermal amplification (RT-LAMP). Step 1: At the 3′-end of the viral RNA, reverse transcriptase and BIP primer initiate conversion of RNA to cDNA. Step 2: At the same end, DNA polymerase and B3 primer continue to generate the second cDNA strand to displace and release the first cDNA strand. Step 3: The FIP primer binds to the released cDNA strand and DNA polymerase generates the complementary strand. Step 4: F3 primer binds to the 3′ end, and DNA polymerase then generates a new strand while displacing the old strand. LAMP cycling produces various sized double-stranded looped DNA structures containing alternately inverted repeats of the target sequence as detected by a DNA indicator dye. Reagents*: Primers and master mix containing reverse transcriptase, DNA polymerase with strand displacing activity, dNTPs.

While RT-PCR is the largely used means for finding COVID19 infections but it has requiring high-priced laboratory instrumentation with highly skilled laboratory staff, and may take more than 24 Hours to produce results. Due to these disadvantages various medical companies and laboratories around the world are continuously working to improve the efficiency and reduce processing time of the RT-PCR technologies and also develop various other techniques.

c. Isothermal Nucleic Acid Amplification.

Multiple temperature changes are required in RT-PCR for each cycle therefore involving complicated thermal cycling apparatus. Isothermal nucleic acid amplification is an substitute approach that permit amplification at a stable temperature and remove the

need for a thermal cycler. Therefore, this principle utilize to develop several method. Such as Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP). For diagnosisof SARS-CoV-2, RT-LAMP has been developed as a quick and commercial testing substitute for. As shown in Figure 2, RT-LAMP utilise a set of four target/region specific primers to improve the sensitivity and combines LAMP with a reverse transcription stage to allow for the detection of viral RNA.[28,29]

Limitations: The limitations of PCR are a considerable factor to reflect, in spite of the cost-effectiveness and reliability in the investigation of viral infections. The risk of error is very high while handling, particularly during the pippeting phase, in addition, real-time PCR has a extended process-time (2–5h) by contrast to other methods.[30]

2.Next-generation sequencing: Next-generation sequencing (NGS) is one of the top successes of the present time. Outside genome sequencing for well-established organisms, this allowed identification of new viruses responsible for unknown endemic and pandemic human illnesses such as Influenza to recognise their appearance and spread profiles.[31]

NGS plays a very important role to find out the appearance and middle host of SARS-CoV-2. Hundreds of coronaviruses and SARS-CoV-2 genomes are publicly available for researchers to study the origin of SARS-CoV-2 which were identified with NGS [32]

As compare to dideoxyribonucleotide sequencing NGS delivering highest volume of data with high speed and precision. It's become possible by using nearly the same basic principal with some modification and improvement in automation. Basically NGS have three key steps: sample preparation, sequencing and data analysis. Most of the system which are available commercially mainly differ in their sequencing or reading techniques. Professionally clinical finding of viral infections using NGS is gradually more aiming to supply perfect longer read-length in a shortest time with lower cost. The key components of the of the sequencing process are Bioinformatics. it provide explanation of sequencing output fencing output through computational analysis, and then change it into useful information on species, genotypes and the occurrence of mutations conferring virulence or resistance to antivirals.[33]

Modern technical perspective in data analysis have included with the software reading platforms for immediate recognition of genotypes and mutants of diagnostic importance however, implementation of NGS in clinical settings is increasing, mostly for identify low-abundance drug-resistance patterns like Hepatitis C virus , human immune deficiency virus. Nucleotide sequencing of Hepatitis C sub-genomic part is presently the technique of choice for genotyping.[34]

Limitations: The basic needs for NGS are primarily access to a sequencer, and significant skills in bioinformatics and knowledge in data analysis in addition of enough handling systems for storage of generated data. Adding to that, in spite of the great results delivered by prototypes in trials, many are still at the research level, and not yet approved for use in routineclinicalpractice.[35]

1. Nucleic Acid Hybridization Using Microarray.

Well-organized and sensitive identification of various viruses included SARS-CoV nucleic acids have also been proceed with microarray techniques. In this technique cDNA developed from viral RNA, and then labelled with specific probes. Microarray tray fixed with Solid-phased oligonucleotides are used for loading labelled cDNA. The existence of specific-viral nucleic acid will be exposed if the process of hybridization occurs. The microarray assay effectively detect the mutations and single nucleotide polymorphisms related to the SAR-CoV gene. It would facilitate the rapid detection of different COVID-19 variations. Moveable microarray chips have provided professional identification of the MERS corona virus with addition to influenza and respiratory syncytial viruses. Microarray assay which use a scanner to demonstrate the hybridization between the probe and target are quite rapid, sensitive, specific, and accurate means of detection. While it can identify many samples, analysis of a few viral genes in inadequate samples is not achievable with this technique. [35, 36]

4.Immunoassay and Serological Based Diagnostic Techniques :

Antibodies detection in patient sample is widely used technique for identification of different viral infection. Serological or immunoassay method basically based on the principle of Ag-Ab complex formation. This complex form between Antibody

present in the patient serum and artificial antigen present in the reagent to develop result finding.

Several immune assay develop by using conjugated synthetic antibodies or antigens which are conjugate to a solid phase, and used to capture their corresponding antigens or antibodies available in patient serum sample. These conjugates could various types like radioactive isotopes, enzymes or fluorescent substance which develop in colour or light-generating substances.[37,38]

Radio-immunoassay (RIA) is probably the initiating immune assay develop in 1960s (1960s); In this techniques radioisotopes (such as Iodine 125) use to label antigen or antibody. The result is based on measuring generated radioactivity. This is a highly sensitive techniques but the main disadvantage is the processing and disposal of hazardous radioactive substances. Now EIA in which enzymatic labelling use alkaline phosphatase or horse radish peroxidase, however, the most widely used alternative method. [39] This enzyme-linked immunoassay (EIA) has several variants, including ELISA, these variants differ in enzyme labelled and the detection of signal principle.[40]

For the detection of various viruses included SARS-CoV Immunoassay have vast potential for their epidemiology but test results can be impacted by at least three situations:

(1) A patient with positive result for SARS-CoV-2 from molecular genetics techniques like RT-PCR may be sero negative with immunoassay due to the lag phase for antibody production after infection,

(2) The patient sample may be seropositive with immunoassay which indicate earlier, milder infection even patient sample however negative for molecular genetics assay .

(3) limitation in sensitivity and specificity of the assays is particularly important because even a small percentage of false positive results due to low cross reaction (low specificity) may show to confusing predictive antibody prevalence between a given population, which may have unwanted impact on the socioeconomic decisions.[41,42]

Table 3 Examples of Serological and Immunological Tests Used to Detect Viral Protein or Antibodies to SARS-CoV-2 Virus[43]

S.N.	test name	test type	manufacturer/organization name	sample source	Ig or protein detected	test result time/additional information	EUAa	country of approval
1	m2000 SARS-CoV-2 assay	chemiluminescentmicroparticle immunoassay	Abbott Core Laboratory	serum/plasma/whole blood	IgG	runs up to 100–200 tests/h		United States
2	COVID-19 IgG/IgM LF	lateral flow immunoassay	Advagen Biotech	serum/plasma/whole blood	IgG/IgM	results in 10 min		Brazil
3	COVID-19 IgG/IgM Point of Care Rapid test	lateral flow immunoassay	Aytu Biosciences/Orient Gene Biotech	serum/plasma/whole blood	IgG/IgM	results in 2–10 min		China, United States
4	COVID-19 IgM/IgG rapid test	lateral flow immunoassay	BioMedomics	serum/plasma/whole blood	IgG/IgM	results in 15 min		United States
5	IgG antibody test kit for novel coronavirus 2019-nCoV	magnetic particle-based chemiluminescence immunoassay	Bioscience (Chongqing) Diagnostic Technology Co., Ltd.	serum	IgG		NMPA	China
6	One-Step COVID-2019 test	lateral flow immunoassay	CelerBiotechnologia	serum/plasma/whole blood	IgG/IgM	results in 15 min		Brazil
7	qSARS-CoV-2 IgG/IgM rapid test	lateral flow immunoassay	Cellex Inc.	serum/plasma/whole blood	IgG/IgM	results in 15–20 min, antibodies specific for N protein	Australia 3/31/2020, US FDA 4/01/2020	Australia, United States
8	COVID-19 Ag Respi-Strip	lateral flow immunoassay (dipstick)	CorisBioconcept	nasal mucus swabs	viral antigen	results in 15 min		Belgium
9	DPP COVID-19 IgM/IgG system	lateral flow immunoassay	Chembio Diagnostics	serum/plasma/whole blood	IgG/IgM	results in 15 min	US FDA 4/14/2020	Brazil

10	DEIASL019/020 SARS-CoV-2 IgG ELISA kit	ELISA	Creative Diagnostics	serum/plasma	IgG/IgM	IgG specific for N protein		United States
11	OnSite COVID-19 IgG/IgMrapid test	lateral flow immunoassay	CTK Biotech Inc. (USA)	serum/plasma/whole blood	IgG/IgM	results in 10 min		Australia
12	Diazyme DZ-Lite SARS-CoV-2 IgG/IgM test	luminescent immunoassay	Diazyme Laboratories	blood sample	IgG/IgM		EUA not required	United States
13	KT-1033 EDI Novel Coronavirus COVID-19 ELISA kit	ELISA	Epitope Diagnostics	serum	IgG/IgM			United States
14	VivaDiag COVID-19 IgM/IgG rapid test	lateral flow immunoassay	Everest Links Pte Ltd.	serum/plasma/whole blood	IgG/IgM	results in 15 min		Singapore
15	COVID-19 IgG/IgM rapid test cassette	lateral flow immunoassay	Hangzhou Biotest Biotech Co. Ltd.	serum/whole blood	IgG/IgM	results in 15–20 min	Australia 4/4/2020	Australia
16	VITROS-Immunodiagnostics Products Anti-SARS-CoV-2 total reagent pack	ELISA	Ortho-Clinical Diagnostics	blood serum/plasma	IgG/IgM	cannot distinguish between IgG/IgM	US FDA 4/14/2020	United States
17	SARS-CoV-2 rapid test	lateral flow immunoassay	PharmACT	whole blood/serum	IgG/IgM	results in 20 min, N protein, S1 and S2 subunits used as antigens		Germany
18	Standard Q COVID-19 IgM/IgG Duo	lateral flow immunoassay	SD Biosensor	serum/plasma/whole blood	IgG/IgM	results in 10 min	EUA not required	South Korea
19	Standard Q COVID-19 Ag	chromatographic immunoassay	SD Biosensor	nasopharyngeal swabs	viral antigen	results in 30 min		South Korea
20	iFLASH-SARS-CoV-2-IgG/IgM	immunoassay	Shenzhen Yhlo Biotech Company	serum/plasma/whole blood	IgG/IgM			China

[a] Emergency Use Authorization by US FDA or other drug regulatory authorities.

4. Biosensors and Nano-Biosensors Based Diagnosis of Viral Infection

Viral Infection Diagnosis Using Biosensors and Nano-Biosensors

Detection of Viral Pathogens In order to prevent outbreaks or pandemics, it is critical to diagnose viral pathogens early and effectively. For that reason, biosensors are being widely applied for making diagnosis easier, bypassing hard proteins or DNA identification approaches in specific virus. [46,47] Because of its ease of dissemination and continual evolution, the influenza virus is one of the most prevalent and lethal viral diseases. As a result, early detection can be challenging. [48,49]. For ultrasensitive and selective Haemophilus influenza detection in human biofluids, Hassanpour et al. developed a new optical biosensor constituted of pDNAbioconjugated citrate capped AgNPs towards target sequences[50]. This pathogen has also been discovered using various biosensors, according to Jiang et al.[51,53,54]. The research reports the invention of a polydiacetylene sensitive biosensor that detects H5N1 (avian influenza) utilising antibody detection, in which the polydiacetylene vesicles change colour from blue to red when the virus is detected. Lee et al. also developed a label-free localised SPR biosensor for the detection of H5N1 with a LOD of 1 Pm (i.e. 1012 M) in a different way. The device, on the other hand, was made with a multifunctional DNA three-way junction mounted on a hollow Au spike-like NP. The bioprobe displayed adequate target recognition and signal amplification capabilitie[55,56]. Other dangerous viruses that affect the population worldwide include ebolavirus, HIV, and Hantavirus [57,58]. The first one is a negative strand-RNA virus that belongs to the

Filoviridae family and causes a deadly disease called Ebola[59]. The infected people with this agent develop a series of symptoms, where hemorrhagic fever is considered as fatal .[61,62,63] Currently, there is no vaccine or specific treatment. However, different studies have presented the development of biosensors for detecting this pathogen[64]. Ilkhani et al. fabricated a novel electrochemical-based-DNA biosensor through enzyme-amplified detection to improve the sensitivity and selectivity of the device for the pathogen[65]. Baca et al. also created a biosensor that can detect the virus within 10 minutes at the POC using surface acoustic waves, indicating that it could be detected before symptoms appear HIV, on the other hand, is a retrovirus that targets a patient's immune system, making them susceptible to a variety of diseases and eventually leading to death if not treated.[66] Clinical HIV treatments are critical for lowering mortality, but early detection can also save lives and reduce transmission rates67,68,69. When the biological analyte binds to the biosenso, Shafiee et al. developed a photonic crystal biosensor that can detect several HIV-1 subtypes (A, B, and D).[70] Furthermore, Gong et al. used reverse-phase polymerization to create a polyaniline/graphene (PAN/GN) nanocomposite for the construction of an electrical DNA-biosensor with high selectivity and sensitivity for the detection of HIV-1 gene fragment[71].

Hantavirus is a cluster of viruses that are part of the Bunyaviridae family. The spread begins through contact with liquids, food, or particles contaminated with rodent excreta. It causes hemorrhagic fever, respiratory insufficiency, and heart failure within 2–7 days after getting infected[72,73]. Regarding its detection, Gogola et al. have achieved significant examination for the development of biosensors [74,75]. In a first methodology, they prepared an electrochemical immune sensor based on chemical alteration of the gold surface with the viral protein[76]. In a second study, the research group designed a quick electrochemical biosensor based on biochar (BC) as a carbonaceous platform for immunoassay uses due to its extremely functionalized surface for covalent binding with biomolecules [78]. Both studies developed devices as promising and suitable tools for hantavirus clinical detection[79,80].

In addition, numerous bio-elements can be combined into a biosensor for detection of virus including markers, RNA, structural proteins, and enzymes from the viral pathogens[81]. COVID-19 Pandemic Currently, many viruses are being considered to have the capacity of causing future pandemics. Diverse factors such as fast distribution, a high transmission rate of new variants, difficulties to develop effective and practicalanalytic techniques, as well as the lack of exact vaccines and harmless drugs for treatment management, make them one of the chief threats for mankind[82,83]. The latestsituation is the COVID-19 declared as a pandemic on March 13th, which is an infectious disease with rapid human-to-human transmission caused by SARS-CoV-2. This pathogen belongs to the positive-strand RNA viruses[84,85]. Like any other viral outbreak, an early diagnosis is fundamental for preventing an uncontrollable spread of the disease. However, this pandemic has the particularity that more than 30% of the confirmed cases are asymptomatic, thus making it harder to control [206–208]. RT-PCR is the most used suitable and reliable method for detecting SARS-CoV-2 infections until now. Nevertheless, the technique is time-consuming, labour-intensive, and unavailable in remote settings [86,87]. Although several other methods can be employed for that purpose, such as immunological assays, thoracic imaging, portable X-rays, or magnification techniques, the pandemic spread of COVID-19 demands to develop POC devices for fastdiscovery[88]

Sheridan conditions that there are two types of fast POC biosensors for COVID-19 finding. In the first place, there is a nucleic acid test, which comprises of identifying the virus in the patient’s sputum, saliva, or nasal secretions[89,] The other type usually detect immunoglobulin that is proceed through the examination of blood samples collected after five days of the initial infection, the production of IgM and IgG as a result of immune response due to the presence of the virus. The manufacturing area has established some appropriate POC biosensors for the qualitative finding of SARS-CoV-2 IgM and IgG Immunoglobulin using specimen as low as 10 µL of human serum, venous blood, or capillary blood, finding quick results within 10–15 min. Many of these rapid Ag-Ab tests are paper-based biosensors that achieve a colorimetric adjacent flow immunoassay. In this method, SARS-CoV-2 specific antigens are typically labelled with gold, and fix the parallel host immunoglobulin, which transfer through

a bonding pad. As anti-SARS-CoV-2 IgM antibodies interact with fixed anti-IgM secondary antibodies on the M line, while IgG antibodies interact with anti-IgG antibodies on the G line. Therefore, M or G lines only appear if the specimen contains SARS-CoV-2 exact antibodies, if not, only the control line (C) will be appear. While the use of serological tests to identify SARS-CoV-2 is quiet under debate, these are predicting as crucial tools for the implementation or ceasing of lockdowns established worldwide[90]

In another methodology, Qiu et al. established a plasmonic biosensor that combines the plasmonicphotothermal (PPT) effect and LSPR sensing transduction. The device is established by a two-dimensional gold nano island functionalized with complementary DNA receptors that can selectively detect specific sequences from SARS-CoV-2 through nucleic acid hybridization. In addition to that, PPT can increase the in situ hybridization temperature, which allows differentiating between two similar gene sequences. This biosensor showed high sensitivity with a lower LOD at 0.22 pM[91]. Although we have discussed several options for COVID-19 diagnosis, researchers are working on novel diagnostic techniques that combine different approaches based on nanotechnology and nanoscience, in order to obtain faster, reliable, and more accurate results that allow accelerating life-saving decisions, and isolation of positive patients in an early stage to down-regulate the virus spread [92].

CONCLUSION AND FUTURE PROSPECTS

The development of diagnostic methods is growing rapidly with lots of modification in well-established techniques. These techniques are remodelling the area of diagnostic microbiology, and possibly will gives the better result to dropping the occurrence of serious infectious diseases. However, the diagnostic abilities are insufficient if healthy promotion is deficient in health sector. For the early detection and screening of infectious patient with accurate and sensitive result is necessary, currently it's the need of diagnostic lab to work on new rapid test kit with high efficiency and accuracy to reduce false positive and negative result.

Whereas the last few months have observed rapid progress in diagnostic kit development for COVID-19, the race continues to develop even more efficient laboratory techniques and cost-effective, point-of-care test kits that can be deployed in mass quantities. This is a requirement to increase the research for rapid diagnosis of current COVID 19 Variants detection.

To promote more accurate and faster diagnostic solutions, a number of organizations are supporting these efforts by inviting assay developers to submit their test products for independent evaluation or by providing huge investments for greater collaboration.

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