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Review Article

Microbiology Section

An Update on the Recent Diagnostic Modalities of SARS-CoV-2: A Review

MARIE VICTOR PRAVIN CHARLES[1], ARUNAVA KALI[2], SANTHANAM VIDYALAKSHMI[3]

ABSTRACT

The pandemic of respiratory viral pneumonia initiated as an outbreak during December 2019, at the Hubei region, China. The demanding research work conducted by the scientific community across the globe identified the causative agent as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The Case Fatality Rate (CFR) is high for the infection. Laboratory diagnosis of infectious diseases has an integral role in case surveillance, diagnosis, treatment and prevention. SARS-CoV-2 is a positive sense single stranded Ribonucleic Acid (RNA) virus grouped under the order *Nidovirales*, family *Coronaviridae*, and subfamily *Orthocoronavirinae*. The structural proteins are spike protein (S), membrane protein (M), nucleocapsid protein (N), and the envelope protein (E). The common symptoms are fever, cough, dyspnoea, fatigue, headache, muscle pain, anosmia and ageusia. The less common symptoms are sore throat, congestion, nausea, vomiting and diarrhoea. Sample collection is a critical step in the laboratory diagnosis of SARS-CoV-2 infection. The molecular methods used for diagnosis of SARS-CoV-2 are Real time Reverse Transcription Polymerase Chain Reaction (r-RT-PCR), Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP), digital PCR, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based assay and sequencing methods. The antigen and antibody detection methods supplement the molecular tests. Although, a huge array of tests are available, the pertinent choice of diagnostic test is crucial. The molecular detection methods are highly sensitive, accurate and reliable.

Keywords: Coronavirus disease 2019, Nucleic acid testing, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

The pandemic of respiratory viral pneumonia initiated as an outbreak during December 2019, at the Hubei region, China [1]. In spite of adherence to strict public health control measures the virus had spread across the globe. The higher reproduction number (Ro) of the virus and transmissibility from asymptomatic individuals had made the infection control measures cumbersome [2]. An infected case can spread to 3-4 individuals with Ro of 3.32 [3]. The demanding research work conducted by the scientific community across the globe identified the causative agent. The phylogenetic RNA sequencing of the virus revealed 79.5%, 50%, and 96% homology with the already existing SARS-CoV, Middle East Respiratory Syndrome virus (MERS-CoV), and bat coronavirus (TG13), respectively [4]. Hence, the virus was named as SARS-CoV-2 by the International Virus Taxonomy Committee (IVTC).

A systematic review conducted in 2022 reveals an estimated Case Fatality Ratio (CFR) of 29% in the Intensive Care Unit (ICU) patients, 15% in hospitalised patients and 1% in the general population [5]. However, the CFR varies based on the geographical region and the wave forms. As per the World Health Organisation (WHO) around 6,418,958 deaths have been reported till August 10, 2022, globally. The highest number of confirmed cases is reported from Europe estimated to be 243,772,549 (42%) and lowest in Africa 9,233,083 (2%) [6].

Laboratory diagnosis of infectious diseases has an integral role in case surveillance, diagnosis, treatment and prevention. It also aids in triage of infected individuals, tracing their contacts thereby curtailing the infectious chain. The management of pandemic warrants optimisation of laboratory testing services across the globe. However, a pragmatic approach on the choice of tests considering the logistics in resource poor settings was the absolute necessity. The molecular tests which were considered gold standard alluded such concerns. In addition, the impediment was on designing, validation and approval of the diagnostic tests. The choice of apt diagnostic test befitting clinical setting mystifies the treating physician. Since, the sensitivity and specificity of the different diagnostic tests varies. A proper choice of laboratory test will guide in the early therapeutic intervention. The aim of this review was to discuss the recent diagnostic modalities available for the diagnosis of SARS-CoV-2 infection.

VIROLOGY OF COVID-19

SARS-CoV-2 is a positive sense ssRNA virus grouped under the order *Nidovirales*, family *Coronaviridae*, and subfamily *Orthocoronavirinae* [7]. Among the four genera (α, β, γ, and δ) of the subfamily, SARS-CoV-2 belongs to the beta-coronavirus genus (subgenus sarbecovirus). It is a spherical enveloped virus with nucleocapsid of helical symmetry and diameter of 60-140 nm [1]. The envelope contains club shaped spike which gives the virus sun ray appearance. The novel coronavirus emerged because of genetic recombination within the species of the same and related genera [8]. The genome is 32 kb in size and has homology with the SARS-CoV, MERS-CoV, and RaTG13 bat CoV [9]. The genome of the virus has six Open Reading Fragments (ORF) of which ORF 1a/b located at the 5' end encodes 16 nonstructural proteins {NSP1-16 including Ribonucleic Acid (RNA) dependent RNA polymerase and helicase}. Similarly, ORF located at the 3' end encodes four structural proteins [10]. The structural proteins are spike protein (S), membrane protein (M), nucleocapsid protein (N), and the envelope protein (E). The *S* protein has S1, S2 and the Receptor Binding Domain (RBD) which recognises the host cell receptor (ACE-2) and mediates endocytosis, the M protein gives shape to the virus, N protein involves in genome package, and E protein helps in virus assembly and release [11]. In recent times, the emerging newer Variants Of Interest (VOI) and Variants Of Concern (VOC) pose a potential risk to the community [12]. These variants arise because of mutation in the spike protein or the RBD. The variants are highly transmissible, evade the neutralisation by antibodies thereby have a reduced therapeutic response. The VOI are Epsilon (B.1.427, B.1.429), Zeta (P.2), Eta (B.1.525), Theta (P.3), Iota (B.1.526), Kappa (B.1.617.1), Lamba (C.37), Mu (B.1.621). The [Table/Fig-1] shows the variants that are being identified in different regions.

CLINICAL PRESENTATION

The infection is acquired through inhalation of respiratory droplets or contact with the fomites. The incubation period ranges from 11-14 days with a median of five days [13]. The common symptoms are

VOC	Mutation	Effect
Alpha (B.1.1.7 lineage) UK	17 mutations with 8 spike mutation (Δ69-70 deletion, Δ144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H)	Increased affinity to ACE2 receptor, 45-71% transmissibility
Beta (B.1.351) South Africa	9 mutations in spike protein and 3 mutations in RBD (18F, D80A, D215G, R246I, K417 N, E484K, N501Y, D614G, A701V, K417 N, E484K, and N501Y)	Increased affinity to ACE2 receptor, 55% transmissibility
Gamma (P.1) Brazil	10 mutations in the spike protein and 3 mutations in the RBD (L18F, T20 N, P26S, D138Y, R190S, H655Y, T1027IV1176, K417T, E484K, N501Y, L18F, K417 N, E484K)	Increased affinity to ACE2 receptor, increased transmissibility
Delata (B.1.617.2) India	10 mutations in the spike protein (T19R, G142D*, 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N)	Increased transmissibility around 50% more than the alpha strain.
Omicron (B.1.1.529) South Africa	50 mutations with 30 in the spike region.	Increased transmissibility and undetected in the RT-PCR kits.

[Table/Fig-1]: Variants of Concern (VOC) prevalent among the SARS-CoV-2 virus [12].

fever, cough, dyspnoea, fatigue, headache, muscle pain, anosmia and ageusia. Around 89% of hospitalised patients present with fever [14]. The less common symptoms are sore throat, congestion, nausea, vomiting and diarrhoea [15]. The other complications are multisystem inflammatory syndrome in children, acute stroke and myocardial infarction. The median time for the onset of acute respiratory distress syndrome is eight days [16]. Among infected individuals' hospital care is mandated in 15% of cases and critical care is required in 5% of cases [17]. Radiograph is also used as an aid to support clinical suspicion of Coronavirus-2019 (COVID-19). The presence of ground glass opacities in Computed Tomography (CT) scan aids suspicion of COVID-19 with a sensitivity of 97% in comparison to RT-PCR [18]. Similarly, other chest radiograph findings are consolidation, hazy or linear opacities [19]. However, the typical radiographic findings may also be absent in few cases [20].

The other biomarkers which aid diagnosis are elevated aminotransferase levels, lactate dehydrogenase levels, elevated D-dimer levels, altered neutrophil lymphocyte ratio, elevated inflammatory markers such as ferritin, C-reactive protein, procalcitonin, Interleukin (IL)-6 [21]. These markers are considered non specific as their levels are elevated in any infectious disease.

SAMPLE COLLECTION

Sample collection is a critical step in the laboratory diagnosis of SARS-CoV-2 infection. Improper sample collection, storage and transport would alter the results of the laboratory test. Unwarranted practice of this crucial step could produce false negative results altering the sensitivity of the rRT-PCR [22]. Therefore, serial testing with new sample is recommended in cases with strong suspicion [23]. The various samples collected include Upper Respiratory Tract Sample (URTS) such as Nasopharyngeal Swab (NPS), Mid Turbinate Sample (MTS), Oropharyngeal Swab (OPS), saliva and Lower Respiratory Tract Sample (LRTS) such as sputum and Bronchoalveolar Lavage (BAL). Other samples which could be tested are conjunctival swab, stool sample and blood sample. Dacron or polyester swabs are recommended for collection of samples as per the WHO [24]. The positivity of the samples depends on the quality of sample, collection method, the stage of the disease, and the type of the sample. In a systematic review by conducted by Mallett S et al., it was found that the positivity rate was altered based on the duration of the symptoms. It was found 89% at four days postsymptom and 54% at 14 days postsymptom [25].

Upper Respiratory Tract Sample (URTS)

The NPS are collected with the patient head tilted upwards a swab is passed along the nasal cavity to reach the posterior pharyngeal wall. This method may result in false negative results due to improper techniques of sample collection and the patient compliance. Alternatively, OPS and MTS could be collected from the suspected patients. A comparative study of NPS, OPS and MTS conducted by Yu F et al., revealed better results of viral load detection in NPS and MTS compared to the OPS [26].

A non invasive alternative URTS is saliva which could be collected in large volumes and has minimal risk to the healthcare workers [27]. In a study conducted by Pasomsub E et al., the sensitivity and specificity of saliva in comparison to NPS and OPS were 84.2% and 98.9%, respectively. This was less compared to the other URTS [28]. However, the sensitivity was found to improve when a group of people were trained for saliva sample collection (90%) compared to the non trained group (66%) [29].

Lower Respiratory Tract Sample (LRTS)

The LRTS are better option for diagnosis of SARS-CoV-2 compared to the URTS. It is opted when the URTS is negative or the clinical scenario warrants such as ventilated patients. In a study conducted on viral load comparison of 76 positive patients, sputum sample was better compared to OPS and MTS [30]. Similarly, Lin C et al., found that sputum sample had positive rate of 76.9% compared to 44.2% among OPS [31]. BAL specimen is superior to sputum with highest positive rate. Around 93% positive rate was reported from 205 SARS-CoV-2 patients compared to all other samples [32].

Other Samples

The other samples which could be tested are conjunctival swab, stool sample and blood sample. Conjunctival swabs when compared with the NPS had less positive rate irrespective of their ocular symptoms [33]. The detection of SARS-CoV-2 from stool sample was present in patients with and without gastrointestinal symptoms. However, patients with gastrointestinal symptoms had more stool positivity rate (73.3%) compared to the respiratory sample (14.3%) [34]. Asymptomatic patients excrete virus in the stool sample for seven days even after the respiratory sample is detected negative. The SARS-CoV-2 RNA is rarely detected from blood samples of critically ill COVID-19 patients [35].

VIRUS CULTURE

Virus culture is direct evidence for the presence and diagnosis of infection. It lays the fundamental for research studies on virus, newer antiviral drug testing, vaccine development and genomic analysis. The virus was cultured by Harcourt J et al., from the first US COVID-19 patient. The SARS-CoV-2 virus was grown in Vero E6 and Vero CCL-81 cell lines. The cell lines were added with Dulbeco's minimal essential medium, foetal bovine serum, penicillin, amphotericin and trypsin. It was found that comparatively Vero E6 had better results with plaque production in two days [36]. The other cell lines were found to be ineffective in growing the SARS-CoV-2.

A similar effort was made to culture the SARS-CoV-2 from the 12 clinical cases in India. The virus was grown on Vero CCL-81 cell line with Eagle's minimal essential medium, 10% foetal bovine serum, penicillin and streptomycin. On day two, the cell line depicted cytopathogenic effect with syncytium formation. The presence of virus was confirmed by rRT-PCR [37].

MOLECULAR DIAGNOSTIC METHODS

Molecular diagnostic methods are a highly sensitive method compared to the other diagnostic modalities available. However, sensitivity fluctuates based on the stage of the disease, quality of the sample, type of sample, the quality of the reagents, processing method, molecular conditions adapted and the primer-probe set used [38]. The development of primer probe set was based on the global data obtained on the SARS-CoV-2 genomic sequence. The analysis of these sequences has revealed conserved regions in RdRp, E, and N gene which are widely used in molecular diagnosis [4]. The molecular methods used for diagnosis of SARS-CoV-2 are rRT-PCR, RT-LAMP, Digital PCR, CRISPR based assay and sequencing methods.

General Molecular Processing and Precautions

The SARS-CoV-2 samples must be handled with biosafety precautions. The personnel handling the samples must wear personal protective equipment. The laboratory must follow standard protocol for sample handling to avoid aerosol exposure and spillage management. The samples must be handled in biosafety level II or III laboratory. The laboratory must be disinfected periodically with sodium hypochlorite, hydrogen peroxide, or quaternary ammonium compounds to avoid contamination [39]. The biomedical waste must be handled and disposed as per the biomedical waste treatment and disposal guidelines. The laboratory must have separate cabins for donning, doffing, sample receipt, neutralisation, extraction, master mix preparation, template addition and PCR. The standard operating procedures, records of each step followed in the laboratory must be followed as per the laboratory accreditation standards.

Real time Reverse Transcription Polymerase Chain Reaction (rRT-PCR)

The rRT-PCR is a nucleic acid amplification method employing four major steps in the detection process. It includes neutralisation, RNA extraction, conversion of RNA to complementary DNA (c-DNA) by reverse transcriptase enzyme, amplification of specific sites of c-DNA by the DNA polymerase enzyme, and real time optical detection of the amplified products by TaqMan probe or fluorescent dye. TaqMan probe is a hybridised reporter probe which is cleaved by 5' exonuclease activity of polymerase removing the quencher emitting fluorescence. The fluorescent dyes such as SYBR Green could be intercalated with the double stranded DNA [40].

The common targets which are used for rRT-PCR assays are E gene, N gene (N1, N2), S gene, Orf1ab, and the RdRP gene [41]. The RdRP gene and the S gene are highly potential targets which differentiates the SARS-CoV-2 from other coronaviruses [42]. The E gene is present in all the beta-coronavirus and the N gene cross react with other virus of the same group [43]. The choice of the primer/probe set determines the sensitivity and specificity of the assay. The inclusion of RdRp gene and N gene had improved the analytical sensitivity of the assay at 95% detection probability [4]. Similarly, the specificity is less when fluorescent dyes are used compared to the TaqMan probe. This is due to specific targets developed with TaqMan probes compared to the fluorescent dyes.

The other determinants of assay sensitivity and specificity are type of sample, duration of illness at the time of sample collection, viral load, and the lower limit of viral load detection of the PCR kit [44]. These factors can alter the clinical performance of the assay, though it is considered highly sensitive. In a study conducted by Fang Y et al., on 51 symptomatic patients around 15 (29%) had an initial negative result [45]. Similar studies have shown false negative rates of 8 (11%) out of 70 patients and 3.5% (out of 626 patients) [46-48]. A metanalysis conducted by Tsang NNY et al., on sample types had revealed sensitivities of 86%, 85%, and 68% for nasal swab, saliva sample and throat swab, respectively [47]. Another study on the duration of illness had revealed the false negative rates vary as 100% on the first day of symptom, 38% on the fifth day of symptom, 20% on the eighth day of symptom and 66% on the 21st day of symptom [48]. The viral load is higher in presymptomatic patients however lower viral loads may be present at the onset [49].

The Cycle threshold (Ct) denotes the number of cycles required to detect the amplified RNA. The higher the viral load the Ct values are less and vice versa. However, their clinical utility is ambiguous as there are no studies to validate [50]. The unavailability of the quantitative standards for rRT-PCR testing, heterogeneity of the URTS, variable performance efficiencies of assays, and the clinical stage of the illness (symptomatic and asymptomatic) are the factors which hinder the clinical utility of the Ct values. Being a highly sensitive test, the assay results would be positive for the non infectious fragments of virus shed by the recovering patient. Hence, repeat negative test cannot be used for work return policies.

Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP)

The RT-LAMP is an isothermal amplification method in which the nucleic acid is amplified exponentially at a constant temperature (60-65°C). The amplification is performed in an auto cycler with three sets of primers (inner primer, outer primer and loop primer). The target adhesion and extension of the inner and outer primer stem loop structure on either side. The Deoxyribonucleic Acid (DNA) amplification is initiated at the stem loop region and the strand displacement activity of polymerase enables multiple cycles. The amplified products are detected by fluorescence or calorimetric method [51]. The assay has high sensitivity, specificity and less turnaround time compared to the rRT-PCR. In a study conducted by Yan C et al., the sensitivity and specificity of RT-LAMP was 130 (100%) with mean detection time of 26±4 minutes [52]. Similar results of sensitivity and specificity were detected by Huang WE et al., [53].

Other Isothermal Amplification Methods

Other isothermal amplification methods with point of care application are Recombinase Polymerase Amplification (RPA), Rolling Circle Amplification (RCA), Exponential Amplification Reaction (EXPAR), and Exponential Strand Displacement Amplification (ESDA). These methods have less sensitivity except RPA which has sensitivity similar to PCR [54].

Digital PCR

Digital PCR is a newer generation of PCR which helps in quantification of the target genome circumventing standard curve. This method was hypothesised by Sykes PJ et al., in the year 1992 however Voglestein and Kinzler introduced the methodology in 1999 [55]. The sample is divided into different dilutions termed as partitions in individual reaction mixtures. With each reaction mixture containing varying target copies the endpoint fluorescence is identified and Poisson statistics is employed for quantification of the target. Both droplets based digital PCR (dd PCR) and chip based digital PCR (cdPCR) are available. Droplet Digital PCR (ddPCR) employs microfluidics to generate nanoliter sized droplets from the reaction mixture. These droplets are amplified in a thermocycler and the fluorescence emitted is detected in a droplet reader. Similarly, in cdPCR the reaction mixture is separated into compartments in a chip which is amplified in a thermocycler and endpoint fluorescence is detected. The number of targets per partition is estimated by poisson statistics to give the number of copies/microliters [56]. These methods can detect low viral loads present in the samples which are undetected by the RT-qPCR. In a comparative study conducted by Suo T et al., 21 cases were positive by RT-qPCR and 49 cases were positive by RT-dPCR (n=77) [57]. In a similar study conducted by Gniazdowski V et al., around 5.6% of negative specimens were identified by RT-dPCR [58]. Hence, dPCR could be used as an alternative for quantification of viral loads present in the sample.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Based Assay

This method utilises CRISPR RNA (crRNA) and CRISPR proteins (Cas12, Cas13, Cas14) for the detection of target gene. The crRNA binds the target sequence and cuts it followed by collateral cleavage of other surrounding nucleic acids. Cas 12 exhibits collateral activity on DS DNA, Cas 14 exhibits collateral activity on SS DNA and Cas 13 exhibits collateral activity on SS RNA [59]. Each of these cleaved collateral components can be detected by reporters with different signals. The combination of isothermal amplification method with CRISPR is feasible. This aids to discriminate the single nucleotide polymorphism and improves the sensitivity and specificity [60]. Cas12, Cas13 and Cas9 based diagnostic tools have been developed for the detection of SARS-CoV-2. Cas12a based diagnostic tool combining RPA was developed by Chen Y et al., similarly RT-LAMP associated CRISPR lateral flow assay was developed by Broughton JP et al., [61,62].

Gene Sequencing

The newer emerging organism is identified by sequencing methods. These methods aid in the identification, tracing the origin, developing the newer molecular kits and vaccine against the infectious agents. However, each sequencing method has its own limitation and varied sensitivity. Meta-transcriptomics allied with Sanger sequencing aided identification of the first complete genomic sequence of SARS-CoV-2 genome in December 2019 [63]. The sequencing strategies used is shotgun meta-transcriptomics, hybrid capture enrichment, amplicon sequencing and direct RNA sequencing. Shotgun metatranscriptomics is a non culture method of sequencing the RNA from clinical samples. It also employs the illumine platform or the Oxford Nanopore Technology [63]. The amplicon-based sequencing is a target-based analysis which is restricted to specific sequence. Similar targeted sequences which are enriched by hybrid capture is utilised in hybrid capture sequencing. Direct RNA sequencing is allowing detection of complex transcriptional patterns and post-transcriptional modifications in SARS-CoV-2 infection.

ANTIBODY DETECTION METHODS

Rapid diagnostic tests are the need of hour in early diagnosis of SARS-CoV-2 infections. However, cross reaction with other coronavirus limits the diagnostic utility of these tests. The detection methods utilise respiratory, serum and fecal samples for diagnosing SARS-CoV-2 infection. The dynamics of infection decides the positive rate for antibody detection methods. The positivity rate of both IgM and IgG increases 10 days postdisease onset however, IgM declines after 35 days [64]. The presence of IgM denotes early infection and IgG denotes late stage of infection. Hence, identification of the same will help in differentiation of active cases from those who recovering. A systematic review evaluated 38 studies and identified that the IgM and IgG detection rate were 23% and 30% in first week, 58% and 66% in the second week, 75% and 88% in the third week [65]. The other factor that determines the positivity rate is the type of assay. The lateral flow assay is comparatively less sensitive than Enzyme-linked Immunosorbent Assay (ELISA) and chemiluminescent assay [66].

Enzyme Linked Immunosorbent Assay Method (ELISA)

The IgM and IgG antibody detection test utilising Rp3 nucleocapsid protein which has homology to antigens of other SARS related virus was developed [67]. Similarly, antibody detection kits were developed utilising spike protein and nucleocapsid protein [68]. The spike protein-based ELISA kits had better sensitivity compared to the nucleocapsid protein based ELISA kits.

Immunochromatography Test

These tests have a nitrocellulose membrane with immobilised antigen to detect the antibody in patient sample. The presence of control band and test band indicates positivity. The sensitivity and specificity of the rapid immunochromatographic test were 88.6% and 90.63%, respectively [69]. Similarly, Choe JY et al., obtained a sensitivity of 92.9% and specificity of 96.2%, when the rapid immunochromatographic method was compared with RT-PCR [70].

Dried blood spots are also utilised for antibody detection against SARS-CoV-2 S1 protein antigens. The sensitivity and specificity of the same was found to be 100% [71]. Similar other study conducted by McDade TW et al., identified IgG antibodies with a sensitivity of 97% [72].

The serological tests including ELISA, immunochromatographic method, and chemiluminescent assays could be used as an alternative to the molecular tests however their sensitivities and specificities vary depending on the stage of the disease, antibody concentration, antibody specificity [64].

ANTIGEN DETECTION METHODS

The antigen detection methods utilise antibodies against *E, N,* and *S* proteins of the SARS-CoV-2. The duration of illness, type of antigen used, and the viral load are other factors which can alter the sensitivity and specificity of the test [73]. These tests show good results in symptomatic patients during the five days of illness, whereas false negative results occur in symptomatic patients more than seven days of illness. Similarly, the *N* and *S* proteins share homology with other coronaviruses (SARS-CoV-2 and MERS-CoV) providing false positive results. The antigen-based tests are highly specific and less sensitive compared to the Nucleic Acid Amplification Test (NAAT) however, the sensitivity is more during the first week of illness [74]. In a systematic review evaluating 58 antigen tests against NAAT positive SARS-COV-2 patients, the sensitivity was variable showing 72% among symptomatic individuals, 58% among asymptomatic individuals. Among the symptomatic individuals, sensitivity was 78% during first week and 58% during second week [75]. ELISA, chemiluminescence and lateral flow formats are available for diagnosis of SARS-CoV-2. A lateral flow strip format with N protein as target with Limit Of Detection (LOD) of 65 ng/mL [76]. A nano chemiluminescence test targeting spike protein had LOD of 0.1 ng/mL, and a sandwich ELISA test targeting spike protein with LOD 63-500 pg/mL has been developed for antigen detection of SARS-CoV-2 [77]. Antigen test are advantageous detecting mutants of SARS-CoV-2. Incorporation of ACE-2 receptor with spike protein aids in detection of mutants of SARS-COV-2 compared to the amplification tests [78]. A judicious use of antigen tests during the early course of illness is beneficial. However, combination of molecular tests with antigen tests increases the positivity rate.

BIOSENSOR DETECTION METHOD

Biosensors are biotechnology based analytical technique which has gained importance in the field of clinical diagnosis. It has gained importance in the diagnostic field since its discovery by Clark and Lyons in the year 1962 [79]. The bioreceptors, transducer and the signal processing system are the integral components of the system [80]. The bioreceptors such as nucleic acids, glycan, lectin, enzyme, monoclonal antibody, or tissue interact with a biochemical marker/target in the sample analyte. These are converted to a measurable signal (electrochemical, optical, or piezoelectric) by a transducer. These signals are amplified to detect the pathogens of concern qualitatively or quantitatively [81]. The viral biosensors used are piezoelectric, electrochemical, thermal and optical sensors. These viral biosensors are found to be better alternatives as they are inexpensive, rapid, sensitive and portable compared to the conventional methods [82]. A gold nanoparticle-based colorimetric test was developed by Moitra P et al., with LOD of 0.18 ng/µL [83]. A graphene transistor-based biosensor detecting spike antibodies with LOD 242 copies/mL was developed by Seo G et al., [84]. A 3D nano printing platform was developed by Ali MA et al., with LOD 2.8×10^{-15} M for spike protein [85]. A polymer-based biosensor platform with molecular imprinting was developed by Raziq A et al., had LOD of 15fM [86]. A rapid microfluidic biosensor was developed by Lin Q et al., which can detect the IgG, IgM and antigen of SARS-CoV-2 in 15 minutes [87]. Similarly, Broughton JP et al., developed CRISPR/CAS-12 integrated in microfluidics [62]. RT-LAMP has been integrated in microfluidics with LOD of two RNA copies per reaction in 70 minutes.

CONCLUSION(S)

All clinically suspected symptomatic patients and close contacts of asymptomatic individuals must be screened for the detection of SARS-COV-2. Although, a huge array of tests are available, the pertinent choice of diagnostic test is crucial. The molecular detection methods are highly sensitive, accurate and reliable. However, they require trained personnel, instruments and strict quality control. At the contrary antigen and antibody detection methods are less time consuming, do not require sophisticated equipment's, and trained personnel. However, the sensitivity of the antigen and antibody

tests vary based on the dynamics of infection, the viral load, the individual response and the type of assay. The newer diagnostic methods introduced require evaluation before they are used in routine diagnosis. The future research should focus on improving and evaluating the other diagnostic methods and improve the sensitivity and accuracy.

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REFERENCES

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China. N Engl J Med. 2020;382(8):727-33.
[2] Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Trav Med. 2020;27:taaa021.
[3] Alimohamadi Y, Taghdir M, Sepandi M. Estimate of the basic reproduction number for COVID-19: A systematic review and meta-analysis. J Prev Med Public Health. 2020;53:151. https:// Doi.org/10.3961/jpmph.20.076.
[4] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045.
[5] Alimohamadi Y, Tola HH, Abbasi-Ghahramanloo A, Janani M, Sepandi M. Case fatality rate of COVID-19: A systematic review and meta-analysis. J Prev Med Hyg. 2021;62(2):E311-20.
[6] World Health Organization. Weekly epidemiological report on COVID-19. Edn.104. 10 August 2022. https://www.who.int/publications.
[7] WHO situation report-111. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200510covid-19-sitrep-111.pdf?sfvrsn=1896976f_2. Accessed May 10, 2020.
[8] Loomba P, Wattal C, Chakravarti A, Dutta S, Chabbra M, Kale P, et al. Can the march of COVID-19 be halted. Indian J Med Microbiol. 2020;38(1):128-33.
[9] Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Sourvinos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infect Genet Evol. 2020;79:104212.
[10] Yadav R, Chaudhary JK, Jain N, Chaudhary PK, Khanra S, Dhamija P, et al. Role of structural and non structural proteins and therapeutic targets of SARS-CoV-2 for COVID-19. Cells. 2021;10:821.
[11] Fehr AR, Perlman S. Coronaviruses. An overview of their replication and pathogenesis. In: Therapeutic Antibodies, 2015;ume 1282. Berlin/Heidelberg, Germany: Springer. Pp. 1-23.
[12] WHO COVID-19 weekly epidemiological update vol. 74. World Health Organization; January 2022.
[13] Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: Implication of multiple shedding routes. Emerg Microbes Infect. 2020;9:386-89.
[14] Guan WJ, Ni ZY, Hu Y, Liyang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708-20.
[15] CDC. Symptoms of Coronavirus (COVID-19). National Center for Immunization and Respiratory Diseases. 2020. Updated Oct. 26, 2022. Available online at: https://www.cdc.gov/ coronavirus/2019-ncov/symptoms-testing/symptoms.
[16] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalised patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA. 2020;323:1061-69.
[17] Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China. JAMA. 2020; 323:1239-42.
[18] Shi H, Han X, Jiang N, Cao Y, Osamah A, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study. Lancet Infect Dis. 2020; 20:425-34.
[19] Cleverley J, Piper J, Jones MM. The role of chest radiography in confirming covid-19 pneumonia. BMJ. 2020;370:m2426.
[20] Wong HYF, Lam HYS, Fong AHT, Leung ST, Chin TWY, Lo CSY, et al. Frequency and distribution of chest radiographic findings in patients positive for COVID-19. Radiology. 2020;296:E72-78.
[21] Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci. 2020;57:338-99.
[22] Wang X, Tan L, Wang X, Liu W, Lu Y, Cheng L, et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients who received tests with both specimens simultaneously. Int J Infect Dis. 2020; 94:107-09.
[23] Lippi G, Simundic AM, Plebani M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin Chem Lab Med. 2020;58(7):1070-76.
[24] World Health Organization. Laboratory testing for Coronavirus disease (COVID-19) in suspected human cases: Interim guidance. 19 March 2020. https://apps.who.int/.
[25] Mallett S, Allen AJ, Graziadio S, Taylor SA, Sakai NS, Green K, et al. At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. BMC Med. 2020;18(1):346.
[26] Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, et al. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis. 2020;71(15):793-98.
[27] Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med. 2020;383(13):1283-86.
[28] Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, Suksuwan W, et al. Saliva sample as a non invasive specimen for the diagnosis of coronavirus disease 2019: A cross-sectional study. Clin Microbiol Infect. 2021;27(2):285.e1-e4.
[29] Kojima N, Turner F, Slepnev V, Bacelar A, Deming L, Kodeboyina S, et al. Self-collected oral fluid and nasal swab specimens demonstrate comparable sensitivity to clinician-collected nasopharyngeal swab specimens for the detection of SARS-CoV-2. Clin Infect Dis. 2020;73(9):e3106-09.
[30] Martinez RM. Clinical samples for SARS-CoV-2 detection: Review of the early literature. Clin Microbiol Newsl. 2020;42(15):121-27. Doi: 10.1016/j.clinmicnews.2020.07.001. Epub 2020 Jul 23. PMID: 32834295; PMCID: PMC7377694.
[31] Lin C, Xiang J, Yan M, Li H, Huang S, Shen C. Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov-2)-infected pneumonia (COVID-19). Clin Chem Lab Med. 2020;58(7):1089-94.
[32] Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020;323(18):1843-44.
[33] Huang Y, Chen S, Yang Z, Guan W, Liu D, Lin Z, et al. SARS-CoV-2 viral load in clinical samples from critically ill patients. Am J Respir Crit Care Med. 2020;201:1435-38.
[34] Han C, Duan C, Zhang S, Spiegel B, Shi H, Wang W, et al. Digestive symptoms in COVID-19 patients with mild disease severity: Clinical presentation, stool viral RNA testing, and outcomes. Am J Gastroenterol. 2020;115(6):916-23. Doi: 10.14309/ajg.0000000000000664. PMID: 32301761; PMCID: PMC7172493.
[35] Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, et al. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated Interleukin 6 level in critically ill patients with coronavirus disease 2019. Clin Infect Dis. 2020;71(8):1937-42. Doi: 10.1093/cid/ciaa449. PMID: 32301997; PMCID: PMC7184354.
[36] Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, et al. Isolation and characterization of SARS-CoV-2 from the first US COVID-19 patient. bioRxiv [Preprint]. 2020; Mar 7:2020.03.02.972935. Doi: 10.1101/2020.03.02.972935. PMID: 32511316; PMCID: PMC7239045.
[37] Sarkale P, Patil S, Yadav PD, Nyayanit DA, Sapkal G, Baradkar S, et al. First isolation of SARS-CoV-2 from clinical samples in India. Indian J Med Res. 2020;151(2&3):244-50. Doi: 10.4103/ijmr.IJMR_1029_20. PMID: 32362649; PMCID: PMC7366528.
[38] Mardian Y, Kosasih H, Karyana M, Neal A, Lau CY. Review of current COVID-19 diagnostics and opportunities for further development. Front Med (Lausanne). 2021;8:615099. Doi: 10.3389/fmed.2021.615099. PMID: 34026773; PMCID: PMC8138031.
[39] Centers for disease control and prevention. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). December 13 2021. https://www.cdc.gov/coronavirus/2019.
[40] Malewski T, Malewska A, Rutkowski R. RT-PCR technique and its applications. State-of the-art. Journal of Animal and Feed Sciences. 2003;12:403-16. Doi: 10.22358/jafs/67719/2003.
[41] Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, et al. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR assay validated in vitro and with clinical specimens. J Clin Microbiol. 2020;58(5):e00310-20. Doi: 10.1128/JCM.00310-20. PMID: 32132196; PMCID: PMC7180250.
[42] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-73. Doi: 10.1038/s41586-020-2012-7. Epub 2020 Feb 3. PMID: 32015507; PMCID: PMC7095418.
[43] Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterization and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-574. Doi: 10.1016/S0140-6736(20)30251-8. Epub 2020 Jan 30. PMID: 32007145; PMCID: PMC7159086.
[44] Fomsgaard AS, Rosenstierne MW. An alternative workflow for molecular detection of SARS-CoV-2 – escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020. Euro Surveill. 2020;25(14):pii=2000398.
[45] Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, et al. Sensitivity of chest CT for COVID-19: Comparison to RT-PCR. Radiology. 2020;296(2):E115-17. Doi: 10.1148/radiol.2020200432. Epub 2020 Feb 19. PMID: 32073353; PMCID: PMC7233365.
[46] Long DR, Gombar S, Hogan CA, Greninger AL, OReilly Shah V, et al. Occurrence and timing of subsequent SARS-CoV-2 RT-PCR positivity among initially negative patients. Med Rxiv [Preprint]. 2020;May 8:2020.05.03.20089151. Doi: 10.1101/2020.05.03.20089151. PMID: 32511542; PMCID: PMC7274223.
[47] Tsang NNY, So HC, Ng KY, Cowling BJ, Leung GM, Ming DK. Diagnostic performance of different sampling approaches for SARS-CoV-2 RT-PCR testing: A systematic review and meta-analysis. Lancet Infect Dis. 2021;21:1233.
[48] Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. Ann Intern Med. 2020;173:262.
[49] Mack CD, Osterholm M, Wasserman EB, Petruski-Ivleva N, Anderson DJ, Myers E, et al. Optimising SARS-CoV-2 surveillance in the United States: Insights from the National football league occupational health program. Ann Intern Med. 2021;174:1081.
[50] IDSA and AMP joint statement on the use of SARS-CoV-2 PCR cycle threshold (Ct) values for clinical decision-making. Updated March 12, 2021. https://www.idsociety.org/globalassets/ idsa/public-health/covid-19/idsa-amp-statement.pdf.

[51] Baek YH, Um J, Antigua KJC, Park JH, Kim Y, Oh S, et al. Development of a reverse transcription-loop-mediated isothermal amplification as a rapid early-detection method for novel SARS-CoV-2. Emerg Microbes Infect. 2020;9(1):998-1007. Doi: 10.1080/22221751.2020.1756698. PMID: 32306853; PMCID: PMC7301696.
[52] Yan C, Cui J, Huang L, Du B, Chen L, Xue G, et al. Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. Clin Microbiol Infect. 2020;26(6):773-79. Doi: 10.1016/j.cmi.2020.04.001. Epub 2020 Apr 8. PMID: 32276116; PMCID: PMC7144850.
[53] Huang WE, Lim B, Hsu CC, Xiong D, Wu W, Yu Y, et al. RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. Microb Biotechnol. 2020;13(4):950-61. Doi: 10.1111/1751-7915.13586. Epub 2020 Apr 25. PMID: 32333644; PMCID: PMC7264870.
[54] Zhao YX, Chen F, Li Q, Wang L, Fan C. Isothermal amplification of nucleic acids. Chem Rev. 2015;115(22):12491-45.
[55] Sykes PJ, Neoh SH, Brisco MJ, Hughes E, Condon J, Morley AA. Quantitation of targets for PCR by use of limiting dilution. Biotechniques. 1992;13:444-44.
[56] Dube S, Qin J, Ramakrishnan R. Mathematical analysis of copy number variation in a DNA sample using digital PCR on a nanofluidic device. PLoS One. 2008;3:e2876. Available at: https://Doi.org/10.1371/journal.pone.0002876.
[57] Suo T, Liu X, Feng J, Guo M, Hu W, Guo D, et al. ddPCR: A more accurate tool for SARS-CoV-2 detection in low viral load specimens. Emerg Microbes Infect. 2020;9:1259-68.
[58] Gniazdowski V, Paul Morris C, Wohl S, Mehoke T, Ramakrishnan S, Thielen P, et al. Repeated coronavirus disease 2019 molecular testing: Correlation of severe acute respiratory syndrome coronavirus 2 culture with molecular assays and cycle thresholds. Clin Infect Dis. 2021;73(4):e860-69. Doi: 10.1093/cid/ciaa1616. PMID: 33104776; PMCID: PMC7665437.
[59] van Dongen JE, Berendsen JTW, Steenbergen RDM, Wolthuis RMF, Eijkel JCT, Segerink LI. Point-of-Care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities. Biosens. Bioelectron. 2020;166:112445. Doi: 10.1016/j.bios.2020.112445.
[60] Gootenberg JS, Abudayyeh OO, Kellner, Joung J, Collins JJ, Zhang F. Multiplexed and portablenucleic acid detectionplatform with Cas13, Cas12a and Csm6. Science. 2018;360(6387):439-44. Doi: 10.1126/ science.aaq0179.
[61] Chen Y, Shi Y, Chen Y, Yang Z, Wu H, Zhou Z. Contamination-free visual detection of SARS-CoV-2 With CRISPR/Cas12a: A promising method in the point-of-care detection. Biosens Bioelectron. 2020;169:112642. Doi: 10.1016/j.bios.2020.112642.
[62] Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR–Cas12-based detection of SARS-CoV-2. Nat Biotechnol. 2020;38:870-74. Available at: https://Doi.org/10.1038/s41587-020-0513-4.
[63] Chan JFW, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster. Lancet. 2020;395:514-23.
[64] Russo A, Minichini C, Starace M, Astorri R, Calò F, Coppola N; Vanvitelli COVID-19 group. Current status of laboratory diagnosis for COVID-19: A narrative review. Infect Drug Resist. 2020;13:2657-65. Doi: 10.2147/IDR.S264020. PMID: 32801804; PMCID: PMC7413717.
[65] Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev. 2020;6:CD013652.
[66] Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, HAraoui LP, Johnson JC, et al. Diagnostic accuracy of serological tests for covid-19: Systematic review and meta-analysis. BMJ. 2020;370:m2516.
[67] Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: Implication of multiple shedding routes. Emerg Microbes Infect. 2020;9(1):386-89. Doi: 10.1080/22221751.2020.1729071. PMID: 32065057; PMCID: PMC7048229.
[68] Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, et al. Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. J Clin Microbiol. 2020;58(6):e00461-20. Doi: 10.1128/JCM.00461-20. PMID: 32229605; PMCID: PMC7269413.
[69] Li Z, Yi Y, Luo X, Liu Y, Li S, Sun R, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol. 2020;92(9):1518-24. Doi: 10.1002/jmv.25727. Epub 2020 Apr 13. PMID: 32104917; PMCID: PMC7228300.
[70] Choe JY, Kim JW, Kwon HH, Hong HL, Jung CY, Jeon CH, et al. Diagnostic performance of immunochromatography assay for rapid detection of IgM and IgG in coronavirus disease 2019. J Med Virol. 2020;92(11):2567-72. Doi: 10.1002/jmv.26060. Epub 2020 Jun 9. PMID: 32458479; PMCID: PMC7283626.
[71] Karp DG, Danh K, Espinoza NF, Seftel D, Robinson PV, Tsai CT. A serological assay to detect SARS-CoV-2 antibodies in at-home collected finger-prick dried blood spots. Sci Rep. 2020;10(1):20188. Doi: 10.1038/s41598-020-76913-6. PMID: 33214612; PMCID: PMC7678827.
[72] McDade TW, McNally EM, Zelikovich AS, Aquila RD, Mustanski B, Miller A, et al. High seroprevalence for SARS-CoV-2 among household members of essential workers detected using a dried blood spot assay. PLoS ONE. 2020;15(8):e0237833. Available at: https://Doi.org/10.1371/journal.pone.0237833.
[73] Rong G, Zheng Y, Chen Y, Zhang Y, Zhu P, Sawan M. COVID-19 diagnostic methods and detection techniques: A review. Reference Module in Biomedical Sciences. 2021;B978-0-12-822548-6.00080-7. Doi: 10.1016/B978-0-12-822548-6.00080-7. Epub 2021 Sep 1. PMCID: PMC8409760.
[74] Brümmer LE, Katzenschlager S, Gaeddert M, Erdmann C, Schmitz S, Bota M, et al. Accuracy of novel antigen rapid diagnostics for SARS-CoV-2: A living systematic review and meta-analysis. PLoS Med. 2021;18:e1003735.
[75] Dinnes J, Deeks JJ, Berhane S, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev. 20210;3:CD013705.
[76] Grant BD, Anderson CE, Williford JR, Alonzo LF, Glukhova VA, Boyle DS, et al. SARS-CoV-2 coronavirus nucleocapsid antigen-detecting half-strip lateral flow assay toward the development of point of care tests using commercially available reagents. Anal Chem. 2020;92(16):11305-09. Doi: 10.1021/acs.analchem.0c01975. Epub 2020 Aug 5. PMID: 32605363; PMCID: PMC7409939.
[77] Kyosei Y, Namba M, Yamura S, Takeuchi R, Aoki N, Nakaishi K, et al. Proposal of De Novo antigen test for COVID-19: Ultrasensitive detection of spike proteins of SARS-CoV-2. Diagnostics (Basel). 2020;10(8):594. Doi: 10.3390/diagnostics10080594. PMID: 32823866; PMCID: PMC7459804.
[78] Zhou B, Thao TTN, Hoffmann D, Taddeo A, Ebert N, Labroussaa F, et al. SARS-CoV-2 spike D614G change enhances replication and transmission. Nature. 2021;592(7852):122-27. Doi: 10.1038/s41586-021-03361-1. Epub Feb 26. PMID: 33636719.
[79] Krejcova L, Michalek P, Rodrigo MM, Heger Z, Krizkowa S, Vaculovicova M, et al. Nanoscale virus biosensors: State of the art. Nanobiosensors in Disease Diagnosis. 2015;4:47-66.
[80] Perumal V, Hashim U. Advances in biosensors: Principle, architecture and applications. J Appl Biomed. 2014;12(1):01-15.
[81] Ozer T, Geiss BJ, Henry CS. Review-chemical and biological sensors for viral detection. J Electrochem Soc. 2020;167:037523.
[82] Souf S. Recent advances in diagnostic testing for viral infections. Biosci Horizons Int J Student Res. 2016. https://Doi.org/10.1093/BIOHORIZONS/HZW010.
[83] Moitra P, Alafeef M, Dighe K, Frieman MB, Pan D. Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmonic nanoparticles. ACS Nano. 2020;14:7617-27.
[84] Seo G, Lee G, Kim MJ, Baek SH, Choi M, Ku KB, et al. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano. 2020;14:12257-58.
[85] Ali MA, Hu CS, Jahan S, Yuan B, Saleh MS, Ju E, et al. Sensing of COVID-19 antibodies in seconds via aerosol jet Nanoprinted reduced-graphene-oxide-coated 3D electrodes. Advanced Materials. 2021;33(7):e2006647.
[86] Raziq A, Kidakova A, Boroznjak R, Reut J, Öpik A, Syritski V. Development of a portable MIP-based electrochemical sensor for detection of SARS-CoV-2 antigen. Biosensors and Bioelectronics. 2021;178:113029.
[87] Lin Q, Wen D, Wu J, Liu L, Wu W, Fang X, et al. Microfluidic immunoassays for sensitive and simultaneous detection of IgG/IgM/Antigen of SARS-CoV-2 within 15 min. Anal Chem. 2020;92:9454-58.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
2. Professor, Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
3. Professor, Department of Oral Pathology and Microbiology, Indira Gandhi Institute of Dental Sciences, Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Marie Victor Pravin Charles,
Sri Balaji Vidyapeeth University, Puducherry Cuddalore Main Road,
Pillaiyark Uppam, Puducherry-607402, India.
E-mail: antonieolee@gmail.com

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