VOLUME - 10, ISSUE - 12, DECEMBER - 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Microbiology

RECENT DEVELOPMENTS IN LABORATORY DIAGNOSIS OF COVID-19 INFECTION- AN UPDATE

Ivneet Kour	MD (Senior Resident)
Lipika Singhal	MD(Associate Professor)
Varsha Gupta*	MD, MAMS Professor and Head, Department of Microbiology, Government Medical College and Hospital, Chandigarh – 160 032. India

ABSTRACT Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This new virus and disease were unknown before the outbreak began in Wuhan, Hubei Province of China, in December 2019. The World Health Organization (WHO) initially declared COVID-19 as the global public health emergency on 30th January 2020 and subsequently a pandemic on March 11, 2020. Besides availability of RT-PCR there is need for development of rapid point of care tests with better sensitivity and specificity which helps in early and accurate diagnosis and also aids in containing the spread . This review summarizes various molecular diagnostics methods, technical guidelines, and advanced testing strategies adopted in India for laboratory diagnosis of COVID-19.

KEYWORDS :

CRISPR Based Assays

The discovery and recent advances in the biology of "Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated CAS proteins have led to rapid research expansion in molecular diagnostics. The CRISPR/Cas9-based tools were first used to detect Zika virus in 2016. These tests use a test protocol that is simple to apply and easy to interpret enabling results in relatively lesser time. They have been recently applied to develop practical and sensitive detection assays for human pathogens, including bacteria and virus.[1]

- **SHERLOCK-** CRISPR-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique Using synthetic COVID-19 virus RNA fragments, Researchers have been able to consistently detect COVID-19 target sequences in a range between 20 and 200 aM (10-100 copies per microliter of input).[2] The test can be started with RNA purified from patient samples, as is used for qRT-PCR assays, and can be read out using a dipstick in less than an hour, without requiring an elaborate instrumentation. This platform allows multiplexed, portable, and ultra-sensitive detection of RNA or DNA from clinically relevant samples.[3]
- **FELUDA'-** It is India's first paper-based test strip that has been developed by the Council for Scientific and Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi. It is named after the famous Bengali fictional sleuth 'Feluda' although it is also an acronym for FNCAS9 Editor-Linked Uniform Detection Assay. The CRISPR-based Feluda testing works by combining CRISPR biology and paper strip chemistry. Briefly, Cas9 protein, a component of the CRISPR system, is barcoded to interact specifically with the Sars-CoV2 sequence in the patient's genetic material. The complex of Cas9 with Sars-CoV2 is then applied to a paper strip, where using two lines (one control, one test) make it possible to determine if the test sample was infected with Covid-19. The strip basically will change color and would give results in minutes.[4]
- **DETECTR-** Another CRISPR–Cas12-based assay is called as SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR).[5] This assay performs simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT–LAMP) for RNA extracted from swabs in universal transport medium (UTM), followed by Cas12 detection of predefined sequences of virus particle, after which there occurs cleavage of a reporter molecule with detection of the virus. The DETECTR assay can take approximately 30–40 min and is easily visualized on a lateral flow strip. The SARS-CoV-2 DETECTR assay is considered positive if there is detection of both the E and N genes.[6] CRISPR based technologies have a good potential for application as a fast, accurate and portable diagnostic assay for emerging infectious diseases including SARS-CoV-2.

Whole Genome Sequencing

Whole-genome sequencing (WGS) is a method used to determine the complete DNA sequence of a specific organism's genome.[7] This methodology permits variations in the protein-coding region of any gene that need to be identified. It also determines the order of all the nucleotides in an individual's DNA and can determine variations in any part of the genome.[8] As it's known that viruses have higher mutation rate and their genetic material keeps on changing quickly as they replicate rapidly so the WGS will help in gaining insights into the origins of the virus along with the different types of strains circulating and how it spread across the regions. The genomic resource obtained from this sequencing will also allow, identification of new targets for diagnosis and drugs for covid-19.[9] Full-length genome sequences of SARS-CoV-2 can be downloaded from the GISAID database. Mutations specific to the Indian SARS-CoV-2 viruses were identified by comparing the coding regions with respect to the SARS-CoV-2, Wuhan, China.[10]

The identified coding gene sequences can be exploited to produce synthetic antidote and target specific vaccines through genetic engineering technologies. WGS is also useful for designing target specific vaccines and permit insight on how vaccine and therapy efficiency may vary as the virus evolves. Random-amplification deep- sequencing methods played a major role in the initial identification of SARS-CoV-2.[11] Deep sequencing molecular methods such as next generation sequencing and metagenomics next generation sequencing will continue to be needed to determine future mutations of SARS-CoV-2 but are currently impractical for diagnosing COVID-19 infections.[12] Genome sequencing also helps to understand whether the virus strain that is present in India is unique or is like other strains circulating globally.[13]

Artificial Intelligence (ai)

The RT-PCR test serves as the gold standard for confirming COVID-19 patients in community and hospital set ups. However, this assay suffers from high false-negative rates,

due to many factors, such as sample preparation and quality control. Healthcare delivery requires the support of new technologies like Artificial Intelligence (AI), to fight against COVID-19 pandemic. The potential of AI to aid in this particular battle has been difficult to ignore. Role of AI is critical and can serve as a powerful tool in the fight against the spread of the growing pandemic.[14] AI, in this regard, can be referred as the Natural Language Processing (NLP), Machine Learning (ML), and Computer Vision applications, which use big-data based models for pattern recognition, its explanation along with the prediction. These functions can be of greater interest in recognizing, predicting and developing a treatment plan for the COVID-19 infection, while managing its socio-economic impact.[15]

Indeed, a report released by World Health Organization in February 2020 highlighted the important role that artificial intelligence in preparing data in China's initial response to the outbreak ststing that these new technologies were applied to 'strengthen contact tracing and manage priority populations' that were most at risk from infection. In an effort to quickly curb the spread of the disease, provinces in China capitalized on technology to face up to the challenge of containing the outbreak.

Artificial Intelligence (AI) is an innovative technology which is helpful to fight the COVID-19 pandemic. This technology is helpful for proper screening, tracking and predicting the current and future patients. The major applications of this AI are for early detection and diagnosis of the infection.AI is used for the development of drugs and vaccines, and the reduction of workload of healthcare workers. This technology plays an important role to detect the cluster of cases and to predict where this virus will affect in future by collecting and analyzing all the previous data. AI is one of such technology which can easily track the spread of this virus, identifies the high-risk patients, and is useful in controlling this infection in real-time. It can also predict mortality risk. AI can help to fight this virus by population screening, medical help, notification, and suggestions about the infection control. It can help a physician not only on the treatment part of the patient, but also helps him to control the disease.

Recently AI-empowered applications in COVID-19 mainly include the dedicated imaging platform, the lung and infection region segmentation, the clinical assessment and diagnosis, as well as the pioneering basic and clinical research. By decreasing radiologist efforts and improving reporting times, such AI-enabled solutions help in making timely and accurate diagnosis affordable for everyone.

In India ward-level digital twins that modelled the spread of the disease as a function of the number of proximal contacts, average duration of contacts, people and place characteristics, and population demographics like age, gender, comorbidities, etc, have been designed. Moreover, many commercial products have been developed, which successfully integrate AI to combat COVID-19 and clearly demonstrate the capability of the technology. Major symptoms and test analysis are done with the help of AI with the highest of accuracy.[16]

Following are the applications of AI in COVID-19 pandemic

- Early detection and diagnosis of the infection
- Monitoring the treatment
- Contact tracing of the individuals
- Projection of cases and mortality
- Development of drugs and vaccines
- Reducing the workload of healthcare workers
- Prevention of the disease

BioFire

US Food and Drug Administration authorizes the newly developed BioFire coronavirus disease 2019 (COVID-19) test (BioFire Defense, Salt Lake City, UT, USA) for PCR-based detection of RNA from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in clinical samples. The BioFire RP2.1 Panel (EUA) detects 22 respiratory pathogens, including SARS-CoV-2, to help clinicians quickly rule in and rule out common causes of respiratory illness in about 45 minutes. The BioFire® COVID-19 Test are rapid, fully automated assays for the detection of SARS-CoV-2 in nasopharyngeal swabs. Quick turnaround on a broad menu of pathogens may also help clinicians make vital decisions regarding admission, isolation, cohorting, and additional diagnostic testing.[17]

In a study by Creager et al, the BioFire RP2.1 was used to detect SARS-CoV-2 not only in acute presentation of infection, but also later in the course of disease when viral titers in the nasopharynx wane off. It was also stated that detection tapered off at lower concentrations rather than falling off abruptly; it appears that stochasticity plays a role in viral detection when low levels of virus are present, precluding the ability to determine the significance of slight variations in rates of virus detection in more dilute specimens without a large number of replicates.[18]

Next Generation Sequencing

Having a next generation sequencing diagnostic tool available will continue to expand the testing capabilities. Genetic sequencing will help in monitoring if and how the virus mutates, which will be crucial to our efforts to continue to learn and fight this virus. Next-generation sequencing (NGS) is an especially powerful tool for tracking these mutations, and even monitoring a patient's infection progression and immune response. A key benefit of NGS is the ability to scale. For instance, a sequencing system can simultaneously sequence more than 100,000 samples, even when using identification that focuses on only one or two specific regions from the viral genome and one control region from the human genome. Next-generation sequencing (NGS) provides an effective, unbiased way to identify coronavirus strains and other pathogens without prior knowledge of the organisms. Sequencing was used to identify the novel coronavirus causing COVID-19 (SARS-CoV-2) early in the outbreak. NGS continues to provide public health officials, vaccine and drug developers, and researchers with critical evidence, and allows labs to:[19]

- Determine the source of infection and route of transmission
- Identify and characterize co-infections and the role of complex disease
- Provide information on coronavirus strain typing to monitor viral spread
- Screen targets for possible COVID-19 therapeutics

Comprehensively sequence respiratory pathogens (including coronaviruses and recent flu strains) and antimicrobial resistance alleles.

Biosensors

A field-effect (FET) transistor based biosensing device has been invented recently for detecting SARS-CoV-2 in clinical samples. It is considered to be potentially useful in clinical diagnosis, POC testing, and also as on-site detection. The sensor is fabricated by coating graphene sheets of the FET with a specific antibody against SARS-CoV-2 spike protein. The sensor is able to detect the SARS-CoV-2 spike protein at concentrations of 1 fg/ml in phosphate-buffered saline and 100 fg/ml clinical transport medium, which makes it a highly sensitive immunoassay for COVID-19. The FET sensor is sensitive and specific enough for the SARS-CoV-2 spike protein. This method does not require sample pretreatment and labeling, and the biosensor does not cross-react with

SARSCoV and MERS-CoV antigens due to high specificity to SARSCoV-2 spike protein by the selected antibody.[20]

A robust laboratory network is the need of the hour to curtail the effect of pandemic .With nonspecific clinical presentation amongst the symptomatic and asymptomatic, along with non-specific treatment at present and numerous undergoing vaccines trials , it is clear that an early and accurate diagnosis is crucial for proper and timely management of COVID-19.Both molecular assays and serological assay at the earliest along with other approaches will further strengthen the diagnosis of SARS-CoV-2. Therefore, a combined approach in laboratory methodology along with clinical findings act as an essential guide to catch hold the disease at the earliest.

CONCLUSION

At present, the COVID-19 is an epidemic now and is still spreading, besides availabiltiy of the effective vaccines or drugs. Early diagnosis and social distancing are still the most effective ways to protect the public. To summarize the present diagnostic technologies although RT-PCR is the gold standard but other diagnostic tools are under development or have been approved. Antibody-based serological diagnosis is also available widely and can be used with molecular diagnostic technology to improve the detection accuracy. In addition, these method are also useful for epidemiological information and to provide the data to support public health policy decisions also. Futher sesearch should be focus more on improving the sensitivity, accuracy, and more avalability..

REFERENCES

1. Xiang X, Qian K, Zhang Z, Lin F, Xie Y, Liu Y, et al. CRISPR-cas systems based molecular diagnostic tool for infectious diseases and emerging 2019 novel coronavirus (COVID-19) pneumonia. J Drug Target. 2020 Sep;28(7–8):727–31.
2. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc. 2019 Oct;14(10):2986–3012.
3. Abudayyeh OO, Gootenberg JS, Kellner MJ, Zhang F. Nucleic Acid Detection of Plant Genes Using CRISPR-Cas13. CRISPR J. 2019 Jun 1;2(3):165–71.
4. Azhar Mohd, Phutela R, Ansari AH, Sinha D, Sharma N, Kumar M, et al. Rapid, field-deployable nucleobase detection and identification using FnCas9. bioRxiv. 2020 Jan 1;2020.04.07.028167.
5. 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 Jul;38(7):870–4.
6. Tsang J, LaManna CM. Open Sharing During COVID-19: CRISPR-Based Detection Tools. CRISPR J. 2020 Jun 1;3(3):142–5.
7. Balloux F, Brønstad Brynildsrud O, van Dorp L, Shaw LP, Chen H, Harris KA, et al. From Theory to Practice: Translating Whole-Genome Sequencing (WGS) into the Clinic. Trends Microbiol. 2018 Dec;26(12):1035–48.
8. Ai J-W, Zhang Y, Zhang H-C, Xu T, Zhang W-H. Era of molecular diagnosis for pathogen identification of unexplained pneumonia, lessons to be learned. Emerg Microbes Infect. 2020 Mar 16;9(1):597–600.
9. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host Microbe. 2020 Mar 11;27(3):325–8.
10. Maitra A, Sarkar MC, Raheja H, Biswas NK, Chakraborti S, Singh AK, et al. Mutations in SARS-CoV-2 viral RNA identified in Eastern India: Possible implications for the ongoing outbreak in India and impact on viral structure and host susceptibility. J Biosci [Internet]. 2020 Jan 1 [cited 2021 Sep 14];45. Available from: https://europepmc.org/articles/PMC7269891
11. Chen L, Liu W, Zhang Q, Xu K, Ye G, Wu W, et al. RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. Emerg Microbes Infect. 2020;9(1):313–9.
12. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med. 2020 Apr;26(4):450–2.
13. Yadav PD, Potdar VA, Choudhary ML, Nyayanit DA, Agrawal M, Jadhav SM, et al. Full-genome sequences of the first two SARS-CoV-2 viruses from India. Indian J Med Res. 2020;151(2–3):200–9.
14. Bullock J, Luccioni A, Pham KH, Lam CSN, Luengo-Oroz M. Mapping the Landscape of Artificial Intelligence Applications against COVID-19. jair. 2020 Nov 19;69:807–45.
15. AL-Hashimi M, Hamdan A. Artificial Intelligence and Coronavirus COVID-19: Applications, Impact and Future Implications. In: Alareeni B, Hamdan A, Elgedawy I, editors. The Importance of New Technologies and Entrepreneurship in Business Development: In The Context of Economic Diversity in Developing Countries. Cham: Springer International Publishing; 2021. p. 830–43. (Lecture Notes in Networks and Systems).
16. Vaishya R, Javaid M, Khan IH, Haleem A. Artificial Intelligence (AI) applications for COVID-19 pandemic. Diabetes Metab Syndr. 2020;14(4):337–9.
17. Eckbo EJ, Locher K, Caza M, Li L, Lavergne V, Charles M. Evaluation of the BioFire® COVID-19 test and Respiratory Panel 2.1 for rapid identification of SARS-CoV-2 in nasopharyngeal swab samples. Diagn Microbiol Infect Dis. 2021 Mar;99(3):115260.
18. Creager HM, Cabrera B, Schnaubelt A, Cox JL, Cushman-Vokoun AM, Shakir SM, et al. Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2. J Clin Virol. 2020 Aug;129:104538.
19. Jayamohan H, Lambert CJ, Sant HJ, Jafek A, Patel D, Feng H, et al. SARS-CoV-2 pandemic: a review of molecular diagnostic tools including sample collection and commercial response with associated advantages and limitations. Anal Bioanal Chem. 2021 Jan;413(1):49–71.
20. Seo G, Lee G, Kim MJ, Baek S-H, 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 Apr 28;14(4):5135–42.