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REVIEW OF DIAGNOSTIC METHODS USED FOR COVID -19

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Abstract: Early in December 2019, the human-to-human infectious disease coronavirus disease 2019 (COVID-19) first appeared and is now a serious threat to public health around the world. The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is the culprit behind this respiratory condition. The creation of quick and accurate methods for COVID-19 diagnosis is a crucial step in the fight against infection. To recognize and screen COVID-19 infections, a variety of techniques including genome sequencing, nucleic acid molecular testing, clustered regularly interspaced short palindromic repeats editing technologies, antigen/antibody detection, and computed tomography imaging have been used. Additionally, other cutting-edge diagnostic techniques, like dried blood spots and biosensors, are being created and are listed below. In order to assist researchers in creating timely and efficient technologies to detect this newly emerging virus and its mutations, this publication summarizes the approaches for SARS-CoV-2 detection that are now available.

Index Terms - **syndrome coronavirus type 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), in vitro diagnostics (IVD), Enzyme Immunosorbent Assay (EIA), Nucleic acid amplification tests (NAATs), loop-mediated isothermal amplification (LAMP), N protein in Human Coronaviruses (HCoVs).**

I. INTRODUCTION

COVID-19, caused by the SARS-CoV-2 virus, typically leads to mild to moderate respiratory symptoms in the majority of those infected. The global need for precise tests to detect SARS-CoV-2 has brought significant attention to the field of in vitro diagnostics (IVD). The COVID-19 pandemic has transformed discussions about medicine, with a particular emphasis on IVD and their accuracy in diagnosing the disease. To detect early SARS-CoV-2 infection, healthcare professionals often recommend using oropharyngeal and nasopharyngeal swabs. Quick and accurate diagnostic tests are essential for the effective diagnosis, isolation, and management of COVID-19 patients.[1] Polymerase chain reaction (PCR) has rapidly become the preferred method for confirming SARS-CoV-2 infection due to its adaptability, established reliability, rapid setup, and analytical excellence. Diagnostic solutions for SARS-CoV-2 can either directly identify the virus or assess surrogate markers of infection derived from the host. An array of analytical tools, encompassing molecular, serological, imaging, and chemical approaches, are in development to meet the testing demand. There is a growing interest in point-of-care diagnostics, which offer the potential for swift testing at the patient's location or in community settings. The development of user-friendly diagnostics is crucial for effectively managing the current outbreak and preventing future epidemics.[2,3]The swift global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has given rise to the worldwide COVID-19 pandemic, which has garnered significant global attention. An effective approach to controlling the COVID-19 pandemic involves the development of highly accurate methods for rapidly identifying and isolating individuals infected with SARS-CoV-2. Many companies and research institutions are thus dedicated to creating effective means of swiftly detecting SARS-CoV-2 ribonucleic acid (RNA), antibodies, antigens, and the virus itself. It's important to note that patients with low levels of SARS-CoV-2 RNA may go undetected. Furthermore, there are other potential biomarkers for early screening of COVID-19 patients, such as the NP antigen and SARS-CoV-2 virus particles, but they are found in low levels in bodily fluids, posing a challenge for point-of-care detection. Presently, there are no commercially available antigen or virus detection kits, and the diagnostic value of NP antigen detection in the early stages of infection remains uncertain. Additionally, during the early period of infection, patients often have low levels of antibodies targeting SARS-CoV-2. [3]This leads to the widely-used, but less sensitive, antibody detection method known as CGLFA having a lower detection rate in early COVID-19 patients. Enhanced molecular and serological diagnostic testing is crucial for better patient outcomes and preventing the spread of infection. This is especially important because new data suggests that a significant number of infected individuals exhibit no symptoms, potentially spreading the disease unknowingly. Moreover, rapid diagnostic testing plays a critical role in assessing the

risk associated with reopening workplaces, educational institutions, and other social and cultural establishments.[4,5] Both the public and private sectors are actively working to meet the growing demand for diagnostic testing capacity and the necessary supplies, including swabs, extraction kits, and buffers. Currently, most diagnostic testing for the virus is conducted in centralized laboratories, involving expensive laboratory equipment, time-consuming assays, and trained laboratory technicians. The distinctive genetic characteristics of SARS-CoV-2 allow for high specificity in various diagnostic formats. However, assessing clinical sensitivity is complicated by significant disparities in analytical range and the challenges associated with standardizing these differences.[5]

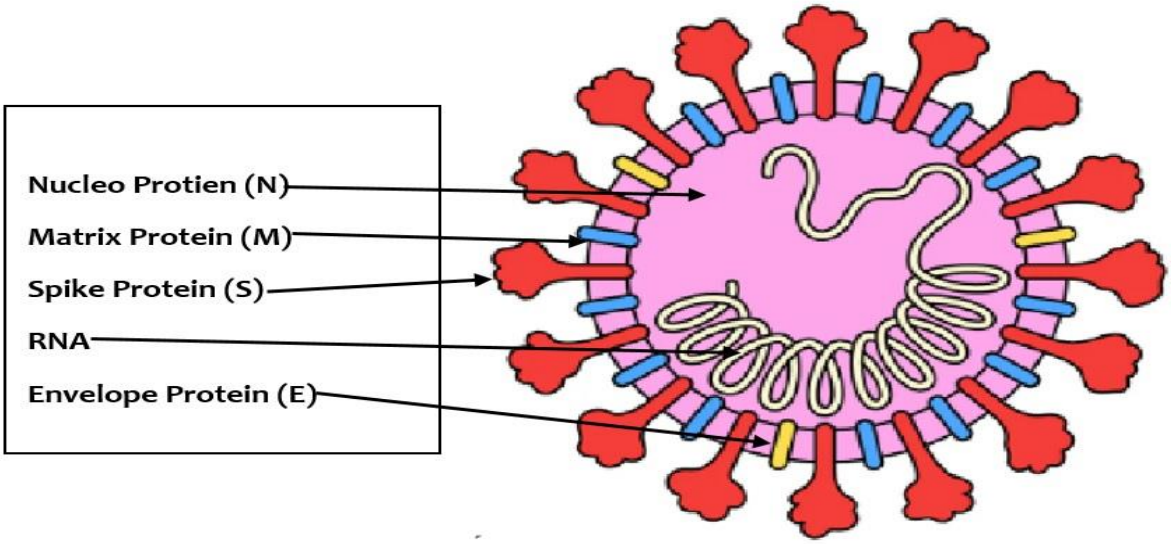

Fig. 1 Structure of sars-cov-2

II. Methods of diagnostic novel solutions for sars-cov-2 associated diagnosis

1.Methods to detect SARS-CoV-2 by using Nucleic acids

2.Detection of Antibodies Against SARS-CoV-2 Proteins by Enzyme Immunosorbent Assay (EIA)

3.Detection of SARS-CoV-2 BY Mass spectrometry

4.Detection of SARS-CoV-2 using biosensors

5. Detection of ANTI-SARS-CoV-2 antibodies

1. **Methods to detect SARS-CoV-2 by using Nucleic acids**

Nucleic acid amplification tests (NAATs), particularly PCR-based methods, are the gold standard for detecting SARS-CoV-2 infection by identifying viral RNA in patient samples. These tests are available in centralized laboratories and point-of-care formats. NAATs, primarily PCR, are commonly used for this purpose. However, NAATs typically require nucleic acid purification, which adds complexity and time to the testing process.

Various specimen types, including respiratory samples obtained with swabs (often combined with viral transport media), saliva, stool, and blood, can be used for SARS-CoV-2 testing.

PCR-based methods, including reverse transcription-polymerase chain reaction (RT-PCR), are widely employed for SARS-CoV-2 RNA detection. Digital PCR is also utilized to quantify viral nucleic acids with high precision and sensitivity-PCR can measure individual RNA molecules, limiting avenues for sensitivity improvements. Isothermal methods like loop-mediated isothermal amplification (LAMP) are proposed as simpler, faster alternatives, particularly suitable for low-resource laboratories. In recent years, NAAT innovation has focused on user-friendly point-of-care formats and higher throughput solutions, as well as advancements in preanalytics. Non-traditional molecular diagnostic techniques like next-generation sequencing (NGS), CRISPR-based assays, and nanotechnology enhance the accuracy and sensitivity of COVID-19 diagnosis.[6]

While RT-PCR is faster, more scalable, and cost-effective, various SARS-CoV-2 NAT kits have emerged in China during the pandemic, mainly based on qRT-PCR technology.

Most qRT-PCR kits can detect viral loads ranging from 100 to 500 copies in a reaction. Consequently, they are suitable for high viral load detection, but results may vary across different kit manufacturers or even between batches from the same manufacturer.[3,4]

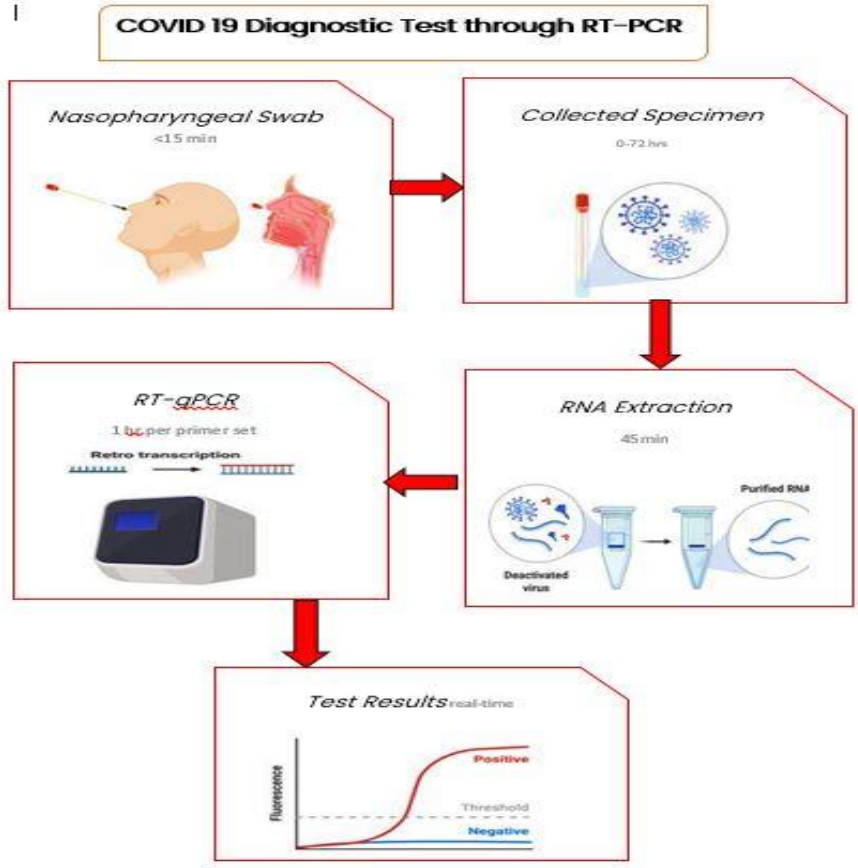

Fig. 2 Steps for RT-PCR test

2. Detection of Antibodies Against SARS-CoV-2 Proteins by Enzyme Immunosorbent Assay (EIA)

ELISA relies on the specific interaction between antigens and antibodies, coupled with enzymatic signal amplification. ELISA assays for SARS-CoV-2 antibodies assess the host's immune response, including IgM, IgG, and IgA, to determine past virus exposure. Blood samples are systematically gathered from individuals to extract serum for use in Enzyme-Linked Immunosorbent Assays (ELISAs). ELISA offers the benefits of speed, sensitivity, simplicity, and ease of standardization. Antibody tests offer a straightforward way to detect SARS-CoV-2 antibodies, allowing for the comparison of multiple samples from a single patient. ELISA-based measurements yield positive detection rates of 85.4% for IgG and 75.6–93.1% for IgM in patients.

This technique is used to quantify proteins, peptides, antibodies, or hormones from biological systems.

The SARS-CoV-2 Spike glycoprotein, responsible for cell attachment and entry, is exposed on the virus's surface and is a critical target for generating neutralizing host antibodies. Consequently, the Spike protein is a central focus of antibody and vaccine development. Enzyme Immunoassays (EIA) are diagnostic methods employed to identify antibodies in patient blood samples or nasopharyngeal swabs.

The N protein in Human Coronaviruses (HCoVs) serves as an antagonist of interferon and a viral-encoded repressor of RNA interference (RNAi). It plays a pivotal role in viral replication and is also a key target for antibody-based therapeutic design. Recombinant antigens derived from the receptor binding domain of the Spike protein and recombinant N protein are in development as suitable diagnostic targets to detect IgM, IgG, and IgA antibodies. Dual detection of IgM/IgG and IgG/IgA immunoglobulins, combined with nucleic acid testing, is in progress for active infection detection and determining previous exposure to SARS-CoV-2.[6,3]

3. Detection of SARS-CoV-2 BY Mass spectrometry

Mass spectrometry identifies viral proteins/peptides by analyzing their mass and charge characteristics. Novel approaches are being explored as potential alternatives to established methods for detecting pathogen-associated proteins. Mass spectrometry spectral analysis is applied to study biomarkers associated with varying levels of COVID-19 severity.[7]

Nucleocapsid and spike proteins are emerging as key targets for SARS-CoV-2 diagnostic detection using mass spectrometry. Clinical settings have successfully utilized mass spectrometry-based proteomic approaches such as liquid chromatography (LC)–MS and matrix-assisted laser desorption/ionization (MALDI)-MS. Mass spectrometry methods have been developed with a reported limit of detection of approximately 200 Amol (about 105 copies/ml) for detecting nucleocapsid and spike proteins in mock samples. While high analytical sensitivity may be less critical in mass COVID-19 testing, mass spectrometry offers potential novel approaches that can complement existing procedures. Ambient ionization and related mass spectrometry techniques enable rapid on-site analysis without complex sample preparation, making them less reliant on advanced laboratory facilities, similar to nucleic acid amplification tests (NAATs). Mass spectrometry provides valuable diagnostic information that complements genomic data, enhancing our understanding of COVID-19 caused by the SARS-CoV-2 virus. An immunoaffinity purification approach followed by a high-resolution mass spectrometry-based targeted qualitative assay is used to detect SARS-CoV-2 viral antigen in

nasopharyngeal swab samples. The nucleocapsid protein is selected as the target antigen based on experiments using purified virus, recombinant viral protein, and clinical samples from COVID-19 patients. Tandem mass spectrometry-based assays for direct detection of infectious agents in clinical samples have the potential to be employed as diagnostic tools in clinical laboratories, expanding beyond the analysis of pure microbial cultures. Mass spectrometry enhances our understanding of COVID-19, caused by the SARS-CoV-2 virus, by providing valuable diagnostic information complementing genomic data.[5-7]

4. Detection of SARS-CoV-2 using biosensors

A biosensor is a specialized device designed for the identification of specific biological substances.

Biosensors comprise three essential components: a recognition element (which interacts with the biological target), the target itself, which translates this interaction into a measurable signal, and electronic components, including an amplifier for signal detection.

When it comes to collecting and processing samples, biosensors prove to be valuable tools for rapidly quantifying biological substances of interest. They find applications in disease diagnosis and environmental monitoring. These biosensors employ a combination of techniques involving nucleic acids or proteins to capture and detect viral proteins and RNA.Various specimen types, such as nasopharyngeal/oral swabs and saliva, have been explored for SARS-CoV-2 detection, but these biosensors are adaptable for use with various samples.[6,7]

Electrochemical immuno-biosensors are capable of identifying viral proteins through changes in electrical conductivity resulting from antibody-antigen interactions. Sensors are comprised of receptors, which can be chemical or biological in nature, and transducers. The receptor interacts specifically with a target analyte, while the transducer converts this interaction into a quantitative signal.[7]

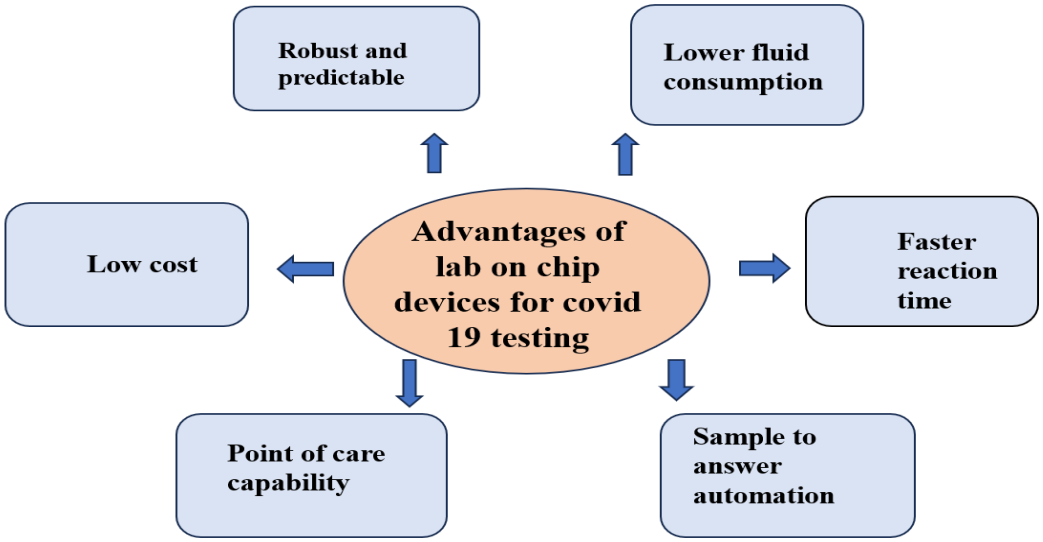

Fig. 3 Advantages of biosensors

5. Detection of ANTI-SARS-CoV-2 antibodies

Serological testing stands apart from direct detection methods as it assesses the host's antibody response, rather than directly detecting the pathogen itself. We've included information on COVID-19 antibody tests here to showcase innovative developments. Traditionally, serology testing has played a crucial role in clinically diagnosing infectious diseases. However, the precise implications of a positive or negative result in the context of SARS-CoV-2 infection are still uncertain.[9]

While serology testing is expected to provide valuable epidemiological insights into SARS-CoV-2, it remains unclear how this relates to immunity, especially considering that antibody levels are reported to decline after six months. Nevertheless, serological testing is likely to play a significant role in assessing the response to future SARS-CoV-2 vaccines.[10]

III. Conclusion

The outbreak of SARS-CoV-2 has prompted the in vitro diagnostics (IVD) community to rapidly create and deploy advanced diagnostic tests for detecting infections. However, it's crucial for the scientific community not to oversimplify this task in their eagerness to offer solutions. A comprehensive approach to implementing new solutions should address both the analytical and clinical challenges associated with SARS-CoV-2 infection while ensuring the reproducibility of the tests. Transmission electron microscopy was employed to identify the virus's structure, genome sequencing confirmed its identity, and sequence data aided in designing PCR primers and probes. This approach will enable the IVD community to leverage various technical disciplines to develop and apply innovative strategies that enhance the accuracy of diagnostic tests for COVID-19, and potentially for other diseases as well. Diagnostics play a vital role in managing outbreaks as they empower healthcare professionals to allocate resources and attention to patients with COVID-19. This process helps contain the spread of infectious agents and reduce mortality rates. These biosensors have the potential to serve as effective tools for swift, reliable, portable, and promising diagnosis during the ongoing pandemic that has had profound impacts on global economies and human health. The current challenges and future prospects of developing robust biosensor devices for rapid, scalable, and sensitive detection and management of COVID-19 are discussed in the context of the World Health Organization's "test-test-test" initiative.

IV.Acknowledgment

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