Redefining Diagnostic Detection: Molecular Diagnostics to LFA technologies

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ABSTRACT

This article gives a comprehensive short overview of diagnostic techniques, emphasizing the transition from conventional methods to advanced molecular, biosensor-based, and lateral flow assay technologies. It highlights the urgent need for sensitive, rapid, specific and effective diagnostic tools to enhance infectious disease control and surveillance. PCR, ELISA, Isothermal amplification and CRISPR-based approaches offer high analytical precision and pathogen detection. This article also outlines the advantages and some limitations of each platform, underscoring the demand for integrated, portable and high-performance diagnostic system.

INTRODUCTION

For the prevention and control of infectious diseases, a rapid and accurate diagnosis is often essential so that a treatment plan can be informedly selected. Timely pathogen diagnosis improves patient outcomes, prevents blind medication, and halts the spread of diseases. The traditional pathogen identification techniques used in clinical laboratories are generally characterized by long test periods and low positive rates. When treating infectious illnesses in particular, a timely diagnosis is even more crucial because it could reduce or prevent further infections in the patient population. Designing techniques that produce data more rapidly while preserving or enhancing sensitivity and specificity is essential since they can play a critical role in

the epidemiology and development of a disease. Therefore, it is necessary to develop highly sensitive, specific, and precise infection management and prevention approaches.

Limiting the source of infection, stopping the disease's means of transmission, and safeguarding people who are vulnerable to infection are the three recommended methods for controlling the infections. One important initial step and important lesson in this approach is the development of targeted, sensitive, and fast detection systems. In the past, diagnosing diseases was mainly based on either identifying the pathogens by microscopy or culturing the pathogens and analyzing the traits of the cultures. However, these approaches alone are not sufficient, since they often yield no findings or are misunderstood, and they take longer to complete (Rajapaksha *et al.,* 2019).

Although there are many approaches to creating diagnostic platforms, modalities founded on optical, electrochemical, magnetic, and colorimetric principles have been thoroughly studied and explored. This article will explore an overview of molecular, biosensors based and lateral flow assays-based diagnosis.

1. Brief overview of Diagnostic methods

The main methods for detecting pathogens include isolation, culture and identification, morphological analysis, gene sequencing, immunological testing, and nucleic acid testing. Only high-level laboratories may use the first two procedures due to differences in pathogen size, culture properties, and infectivity. Accuracy, bioinformatics analysis, and library preparation are still difficult for portable gene sequencers to integrate. As a result, point-of-care testing (POCT) scenarios are more suited for the latter two approaches.

In summary, there are five essential detection principles: microscopic examination, culture and isolation, molecular diagnostics, immunodiagnostics, and histopathology. Each principle and method possesses its unique advantages and disadvantages. Recently, advancements in disease diagnosis have primarily focused on molecular diagnostics, biosensor-based diagnostics, and lateral flow assays (LFAs). Every concept and approach has particular benefits and drawbacks. The main areas of recent progress in disease diagnosis have been lateral flow assays (LFAs), biosensor-based diagnostics, and molecular diagnostics.

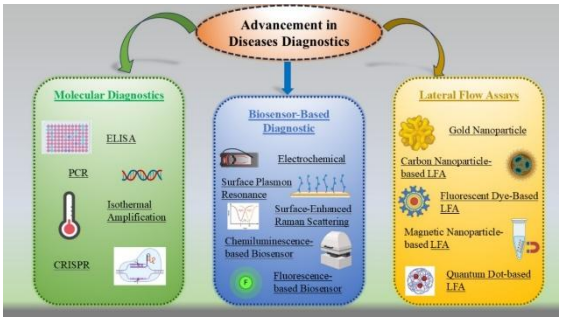

Figure 1. Improvements in the diagnosis of infectious diseases.

2. Molecular diagnostics

Based on the principle of detecting proteins and nucleic acids, a variety of methods are used in molecular diagnostics to examine biological markers present in the genome and proteome. They have remarkable sensitivity and specificity, which help to produce reliable results. These methods become crucial tools for the early identification of infectious diseases because they not only make it possible to detect various pathogens but also make it easier to analyze medication resistance genes and pathogen homology. ELISA, PCR, gene chip techniques, isothermal amplification technology, and high-throughput sequencing technology are most used diagnostic methods.

PCR (Polymerase chain reaction) amplifies particular target nucleic acid sequences. It

undergoes a number of temperature cycles during the procedure, together with free nucleotides and the polymerase enzyme, producing an exponentially amplified target sequence. PCR is renowned for its great sensitivity and specificity, which allow for the accurate detection of a wide variety of bacteria, viruses, and parasites. This method's vast potential has led to modifications and advancements since its introduction. Conventional (end point) PCR, Real-Time (Quantitative/qPCR), Reverse transcriptase PCR (RT-PCR), Digital PCR, Multiplex (multiple targets), Nested (high specificity) PCR, are some of the variants of PCR. Droplet-based systems have also been developed, which use cells in their samples that are transferred onto a microfluidic chip that has different areas for PCR, DNA extraction, purification, cell lysis, and fluorescent signal detection.

ELISA is the most popular technique for the determination and quantification of antigen or antibody in sample. This method is quite versatile and can identify a wide variety of compounds, including bacteria, viruses, and proteins. The use of nanoparticles in ELISA to increase sensitivity and lower the limit of detection (LOD) is one innovation. Direct, indirect, sandwich, and competitive ELISA are among the different types of ELISA that are currently available; they vary in how they apply antibodies and detect targets.

Isothermal amplification techniques, which enable the entire amplification process to take place at a constant temperature, were developed to overcome the drawbacks of PCR settings. Recombinase polymerase amplification (RPA), Nucleic acid sequence-based amplification (NASBA), Rolling circle amplification (RCA), and Loop-mediated isothermal amplification (LAMP) are examples of isothermal amplification. RCA uses a single functional template to amplify target nucleic acids as primers, whereas NASBA, LAMP, and RPA use two or more primers for amplification. Different techniques rely on a variety of different enzymes and proteins in addition to DNA polymerase.

The CRISPR–Cas system, a defense mechanism that bacteria have evolved, is a recently developed technique. When bacteriophages infect bacteria, they pick up CRISPR sequences and as they reinfect, Cas proteins are produced, that subsequently cleaves the viral nuclei acid. Today, it is famous gene-editing technology. It comprises of two groups, and because of their great specificity, these systems have been widely used for gene editing since their discovery. There have also been recent developments about their use in diagnostics.

3. **Biosensors based diagnostics**

Biosensors convert biological reaction signals into optical or electrical impulses (Vidic *et al.,* 2017). For their ability to detect and track a variety of analytes linked to clinical analysis, medical diagnosis, environmental monitoring, and food safety, sensors and biosensors have been extensively studied. They have excellent sensitivity and speed of testing. Fluorescent, colorimetric, SPR-based, SERS-based, electrochemical, and chemiluminescent biosensors are all included in this particular category. Because of their simplicity of use, they can be used as self-testing devices and for point-of-care (POC) testing.

Electrical signals, which can be impedimetric (impedimetric), potential difference (voltammetric), charge (potentiometric), or current (amperometric), are produced by electrochemical (ECL) biosensors from biological reactions. The Zika virus, HIV, Ebola virus, SARS-CoV2, and other infectious diseases have been studied. By utilizing the surface plasmon resonance properties of metallic nanoparticles, surface plasmon resonance (SPR)-based biosensors are

primarily used for the label-free detection of chemicals. These sensors have been used in a wide range of disciplines, such as pharmacokinetics, enzymology, environmental research, and food studies. Biosensors based on surface enhanced Raman scattering (SERS) make use of the Raman scattering concept. Label-free direct methods and label-dependent indirect methods are two ways that SERS-based biosensors make diagnosis easier. The analyte adheres directly to the SERS substrate in the direct technique, and the analyte's distinct Raman fingerprint is used for detection. On the other hand, the indirect method employs tags or reporter molecules to capture the analyte. The changes in the Raman signal of the reporter molecule that is attached to the substrate are then used to detect the analyte.

The fundamental working concept of fluorescence-based biosensors is the emission of fluorescence by a variety of chemicals, including fluorophores, fluorescent nanoparticles, and quantum dots (QDs). A laser with a shorter wavelength stimulates these fluorophores, releasing light energy at a longer wavelength that is subsequently detected by a detector. Iwanaga (2021) developed a biosensor for SARS-CoV-2 identification that uses the nucleic acid hybridization principle.

4. Lateral flow assays (LFAs) based diagnostics

The fundamental idea behind LFAs is the interaction between an antigen and an antibody on a paper strip. Because of their high sensitivity and specificity, low cost, quick findings, and mobility, LFAs are frequently employed as proof-of-concept test devices in low resource environments. LFAs come in two varieties: nucleic acid lateral flow assays, which identify nucleic acids, and lateral flow immunoassays (LFIAs), which identify antigens or antibodies. These tests can be applied to almost every area of diagnosis, including the detection of cancer. Direct and competitive assays are the two forms of LFIAs that are primarily researched. A positive test is indicated in a direct assay when the target analyte binds to the monoclonal antibodies on the test line. In a competitive test, the target analyte in the sample and the tagged detection antibody vie for binding to the immobilized capture antibody. The concentration of the target analyte in the material is inversely correlated with the amount of the labeled detection antibody that binds to the capture antibody. Using an AuNP-labeled recombinant antigen as the detection molecule and anti-human-IgM and anti-human-IgG as capture antigens, Li *et al.* (2020) developed a LFIA to identify IgG and IgM antibodies against SARS CoV-2.

Scientists have created a platform that combines the sensitivity and specificity of CRISPR with the point-of-care potential of LFAs by combining the approaches of CRISPR and LFAs. CRISPR-based LFA diagnostics were thoroughly investigated during and after the COVID-19 pandemic due of its exceptional effectiveness. Ali *et al.* (2022) developed Bio-SCAN, a biotin-coupled selective CRISPR-based assay for nucleic acid detection, to detect SARS-CoV-2.

5. Advantages and Disadvantages of diagnostic techniques

Molecular diagnostics has high sensitivity and specificity; it may be combined with other detection platforms, and, can identify numerous infections at once using a variety of primers (PCR) and antibodies (ELISA). However, it has a number of disadvantages, such as a complicated process, require highly qualified staff, expensive equipment, high testing costs, lack of portability. When using biosensors for diagnostics, little to no sample preparation is required; they are highly sensitive and specific due to their ability to

detect minute changes; they can yield both quantitative and qualitative results; and they are portable and small. They do, however, have certain drawbacks, including the necessity for specialized readers or analyzers, a short stability period, high development costs, frequent calibration, and environmental variables like humidity and temperature.

LFA diagnostics has advantages, including the ability to work as a one-step test, support different sample types, and require a smaller sample amount. It is also suited for point-of-care use, and is portable. However, depending on the detection label used, it can require particular readers or analyzers. Results may vary if the sample volume is changed from the recommended amount. Additionally, the interpretation of colorimetric LFA results might vary from person to person.

CONCLUSION

Technologies that improve global health services are in high demand, especially in the fields of illness prevention, diagnosis, and treatment. Infectious illness diagnostics has undergone a revolution in recent years, marked by the development of new diagnostic procedures and improvements to conventional detection techniques that offer significant advantages over earlier approaches. Diagnostic platforms have advanced significantly as a result of advances in the biological sciences. In addition to these improvements, the growing need for point-of-care (POC) and quick diagnostics has prompted researchers to create novel technologies to overcome these obstacles. In this regard, attempts have been made to improve the portability and reduce the size of detecting platforms.

REFERENCES

Ali, Z., Sánchez, E., Tehseen, M., Mahas, A., Marsic, T., Aman, R., Sivakrishna, R. G., Alhamlan, F. S., Alsanea, M. S., Al-Qahtani, A. A., Hamdan, S. and Mahfouz, M. (2022). Bio-SCAN: A CRISPR/dCas9-Based Lateral Flow Assay for Rapid, Specific, and Sensitive Detection of SARS-CoV-2, *ACS Synthetic Biology*, 11(1):406-419.

Li, Z., Yi, Y., Luo, X., Xiong, N., Liu, Y., Li, S., Sun, R., Wang, Y., Hu, B., Chen, W., Zhang, Y., Wang, J., Huang, B., Lin, Y., Yang, J., Cai, W., Wang, X., Cheng, J., Chen, Z., Sun, K., Pan, W., Zhan, Z., Chen, L. and Ye, F. (2020). Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis, *Journal of Medical Virology*, 92(9):1518-1524.

Rajapaksha, P., Elbourne, A., Gangadoo, S., Brown, R., Cozzolino, D. and Chapman, J. (2019). A review of methods for the detection of pathogenic, *Analyst*. 144(2), 396-411.

Vidic, J., Manzano, M., Chang, C.M. and Jaffrezic-Renault, N. (2017). Advanced biosensors for detection of pathogens related to livestock and poultry, *Veterinary Research*., 48(1), 11.