

REVOLUTIONARY ADVANCES OF DIAGNOSTIC MICROBIOLOGY

Abstract

Recent trends in diagnosis techniques in Clinical microbiology include the development of new methods that can overcome the limitations of traditional culture-based methods, such as long turn-around time and low accuracy. The evolving technologies include: E-Microbiology, Biosensors, Digital droplet PCR, Sequencing, Nanotechnology, CRISPER technology, Lab on Chip. These technologies have evolved due to development of digital technologies and automation in Diagnostic tests. These methods can provide faster and more reliable results, which can help in the management of bacterial infections and sepsis in critically ill patients, especially in the context of increasing antimicrobial resistance. However, these methods also have some challenges, such as high cost, technical complexity, and lack of clinical impact data. Therefore, it is important to understand, integrate and analyze these evolving technologies to evaluate their performance and outcomes in real-life settings

Keywords: E-Microbiology; Biosensor; PCR; NGS; Nanotechnology; CRISPER ;Lab on chip.

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I. INTRODUCTION

The ongoing changes in the spectrum of the infectious diseases with varied pathogenesis and newer emerging microorganisms, has created a great demand for more rapid, simple, reliable diagnostic testing modalities in Microbiology. This is possible with the integration of digitalization and 5G technologies with medical diagnostic modalities. The evolving technologies include: E-Microbiology, Biosensors, Digital droplet PCR, Sequencing, Nanotechnology, CRISPER technology, Lab on Chip. Their application in Diagnosis and limitations with future scope for their development for better patient care, is the current trend in the field of biomedical research.

II. E-MICROBIOLOGY

E- Microbiology is the application of digital technologies to microbiological research and practice. It involves the use of digital devices and methods to acquire, process, analyze, interpret, and share microbiological data. Digital microbiology has several benefits, such as improved data quality, accessibility, and analysis, as well as faster and more accurate diagnosis and treatment of infectious disease. It also has some challenges, such as ethical, legal, and social issues related to data privacy, security, ownership, and governance. Digital microbiology is a rapidly growing field that has the potential to revolutionize the diagnosis and clinical management of the infectious diseases[1].

- Digital Data Acquisition:** This is can be done in each step of Diagnostic process in Microbiology lab [1].

Table 1: E-Microbiology Concepts in Diagnostic Process

Diagnostic Stage	Digital Microbiology Concepts with Examples
Pre Analytical	<ul style="list-style-type: none"> • Automated measurement and feedback regarding the correct filling of blood culture bottles.[2] • Automated assessment of sample contamination and assessment[3]. • Suggestion of Additional supplemental tests from software apps and Artificial Intelligence[4].
Analytical	<ul style="list-style-type: none"> • Automated reagent verification with Internal and External Quality control in surveillance of Lot performance. • Automated Image capturing and analysis for pathogen like structures.[5] • Automated detection of colonies from culture plates and identification (Telebacteriology)[6] • Antibiotic resistant pattern verified by Expert system
Post Analytics	<ul style="list-style-type: none"> • Detection of antibiotic resistance from spectra of MALDI TOF[7] • Prediction of Sepsis and suggests treatment options.

2. **Digital Data Processing:** It includes the techniques and tools for processing digital data from microbiological samples, such as image analysis, signal processing, machine learning, and artificial intelligence. The data storage from these digital platform is one of the challenging aspect. But increase in computer technology paves for the exponential cumulative data storage from all these digital platform.
3. **Digital Data Analysis and Interpretation:** The methods and challenges of analyzing and interpreting digital data from microbiological samples, such as data integration, visualization, quality assessment, and reporting is done by Machine Learning and Artificial Intelligence techniques. It should be trained to collect and interconnect the structured data base to draw a meaningful conclusion. This can be done with or without supervision control.
4. **Digital Data Sharing and Collaboration:** The opportunities and barriers of sharing and collaborating on digital data from microbiological samples, such as data standards, formats, platforms, security, privacy, ethics, and governance is a concern which can be overcome by the consent from patients and institution.
5. **Digital Data Applications and Impact:** The potential applications and impact of digital data from microbiological samples, such as outbreak detection, infection prevention, antimicrobial resistance, diagnosis, treatment, surveillance, and research can be employed appropriately.

III. BIOSENSORS

Biosensors is one of the fast evolving technology to detect rapid and accurate identification of pathogenic organisms. According to the definition of International Union of Pure and Applied Chemistry(IUPAC), biosensors are integrated autonomous devices that provide quantitative(Semi-quantitative) analytical information about the target analyte using a biological recognition element (Bioceptor) that has a spatial relationship with the transducer[8]. In other words, A biosensor is an analytical device that combines a biological component with a physicochemical detector to detect a chemical substance. It typically consists of

1. A bio-receptor (enzyme/antibody/cell/nucleic acid/aptamer)
2. A transducer component (semi-conducting material/nanomaterial), and
3. An electronic system that includes a signal amplifier, processor, and display.

The bioreceptor is designed to interact with the specific analyte of interest to produce an effect measurable by the transducer. The transducer then outputs a measurable signal proportional to the presence of the target analyte in the sample.

One of the classification of Biosensors according to IUPAC based on the detection methods are: with label(Labelled biosensors) and without labels(Label free biosensors)

Labelled biosensors are widely used in biomedical research to detect and quantify various biomolecules replacing the conventional methods. One of the most widely used biosensors is a label-based immunological biosensor. In this type of biosensor, various

techniques and labels have been developed to satisfy the requirements of high sensitivity, high accuracy, and a wide dynamic range. Some of the well known diagnostic tools includes ELISA and Fluorescent biosensors used for detection of proteins, nucleic acids, and small molecules.

IV. CURRENT DEVELOPMENT OF BIOSENSOR

- 1. Label-Free Biosensor:** Label-free biosensors have a promising future in the field of diagnostics. They offer several advantages over traditional laboratory methods, such as real-time detection, high sensitivity and specificity, and cost-effectiveness. These features make them ideal for use in point-of-care testing, where rapid and accurate diagnosis is critical. In the future, label-free biosensors are expected to become more widely used in clinical diagnosis of infectious diseases, environmental monitoring, food safety, and biodefense.

The types of Label free biosensors with its detection mechanism and its application in Infectious diseases with few examples are tabulated in Table 2

2. Diagnostic Tests

Table 2: Label free Biosensors Detection mechanisms & their Applications

Label free Biosensors	Detection Mechanism	Application in Diagnostic Tests
Optical label free Biosensors	Use light to measure changes in refractive index, surface Plasmon resonance, fluorescence, or Raman scattering caused by the binding of biological molecules to a sensor surface.	Avidity of Antibody can be measured in Mycobacterium tuberculosis infection which could be used to determine whether TB vaccine elicit a similar response[9].
Electrochemical label free biosensors	Use electrical signals to measure changes in current, potential, impedance, or capacitance caused by the interaction of biological molecules with an electrode surface.	Detection of S.entericaTyphimurium for quantification which is simple and fast. It can be a great point of care device for evaluating the pathogenicity of unknown bacteria[10].
Microwave label free biosensors	Use electromagnetic waves to measure changes in dielectric properties, permittivity, or conductivity caused by the presence of biological molecules in a sample.	Detection of E.coli in different biological samples like wet biosubstrates, water, or dry environmental objects[11].
Mechanical label free biosensors	Use mechanical forces to measure changes in mass, resonance frequency, or bending caused by the attachment of biological molecules to a micro- or nano-scale structure.	Detection of Prostate specific antigen with wide range of concentration in detection of Prostate cancer[12].

Label-free biosensors face several challenges that blockades its further development. Some of these challenges include:

- **Selectivity:** One of the main challenges for label-free biosensors is to achieve high selectivity, meaning the ability to specifically detect the target molecule in the presence of other similar molecules. This can be challenging in complex biological samples, where many different molecules may be present.
- **Stability:** Another challenge for label-free biosensors is to maintain their performance over time. The stability of the sensor can be affected by changes in temperature, humidity, and other environmental factors, as well as by the degradation of the biological recognition elements.
- **Reproducibility:** Reproducibility is a key requirement for any diagnostic tool, and label-free biosensors are no exception. Ensuring that the sensor gives consistent results over multiple measurements and between different devices can be challenging.
- **Miniaturization:** Miniaturization is important for label-free biosensors to be used in point-of-care testing and other applications where portability is important. Developing sensors that are small, lightweight, and easy to use while maintaining their performance can be challenging.
- **Integration:** Integration of label-free biosensors into existing diagnostic workflows and devices is another challenge. This requires the development of sensors that are compatible with existing equipment and protocols, as well as the development of new protocols and workflows to incorporate the sensors.
- **Standardization:** Standardization is important for ensuring that results from different label-free biosensors can be compared and interpreted consistently. Developing standardized protocols and methods for using and calibrating label-free biosensors is an ongoing challenge.

V. NEWER GENERATION PCR

Polymerase chain reactions have evolved since its first use in 1983. The different generations of PCR [13] are:

- **First Generation PCR:** PCR products are processed with the gel electrophoresis with low detection limit, laborious operational procedures resulting in qualitative analysis.
- **Second Generation PCR** is Real time PCR which can quantifies based on standard curves thereby giving Relative quantification of the analyte.
- **Third Generation PCR** is Digital PCR which produces the absolute quantification of the analyte.

The current trends in development is directed to the third generation PCR. The third generation dPCR partitions the PCR solution into thousands of nanoliter-sized droplets, each containing a separate PCR reaction. By counting the number of droplets that show fluorescence after amplification, dPCR can estimate the absolute number of

target molecules in the original sample. This can be done based on microfluidics either as droplet or chip based.

- 1. Droplet Digital PCR (dd PCR):** The sample is slit into tens to thousands of droplets [14] by an immiscible liquid like mineral oil forming an emulsion. The emulsion is processed for PCR and subjected to flow cytometer to count the droplets with positive PCR. It has the advantages of higher precision in absolute quantification. PCR multiplexing can also be performed by probe-based fluorescence with different excitation colours for different targets. It is also used for single cell RNA sequencing.
- 2. Chip Based Digital PCR:** Silicon chips with wells designed by a micromachining technique is used. PCR is conducted in the chip and imaged by fluorescence microscopy to identify the positive reactions. Multiplexing can also be conducted. Miniaturized PCR system is great boon for easy portability and save time. Researchers have developed rapid PCR cycle, resulting in total reaction time in less than 15 s[15].
- 3. Challenges:**
 - Quality of samples
 - Spectrum of different inhibitors of PCR
 - Purification of sample
 - Structure hindering full denaturation and primer annealing
 - Miniaturized devices.

VI. SEQUENCING[16-17]

Next generation sequencing (NGS) holds an uphold in the diagnosis of many infectious diseases especially viruses. With the cost of sequencing decreasing, shorter turn around time (TAT) and laboratory automation there is an increase in the implementation of the NGS. NGS have been used for strain typing in outbreak investigation, pan pathogen detection and even to detect susceptibility to antimicrobial agents. The three main classes of sequencing that looks promising is the whole genome sequencing (WGS), targeted next generation sequencing (tNGS) and metagenomic next generation sequencing (mNGS).

- 1. Whole genome sequencing:** The whole microbial genome will be sequenced and assembled in this method. The advantages of this method of sequencing are identification of drug resistance (identification of novel resistance, single nucleotide polymorphism, mutational analysis and plasmid mediated resistance). WGS has been used to identification and typing of rare species, and study the different virulence factors. The main limitation of this method of sequencing is in bacterial isolates there is a need pure colony, this requires the growth and isolation of the organism. Though WGS identifies the various mechanism of the drug resistance mainly their expression where profiling of antimicrobial susceptibility pattern will require a reconfirmation with phenotypic methods. There is a higher sequencing depth and cost which is involved in the WGS .
- 2. Targeted next sequencing:** The tNGS is based on target selection to determine the presence or absence of a pathogen and hence decreases or eliminates amplification of unwanted human sequences. Around 300 bacterial species and 200 viruses have been identified by this method till date, many more could be sequences in the future.

The main advantage of this sequencing method is its high sensitivity as targets are selected and it is used as an adjunct where slow growing pathogens are suspected or when patients have previously been exposed to antimicrobials. The additional cost for the enrichment step is a disadvantage of this method.

- 3. Metagenomic Next Generation Sequencing:** The mNGS is a pan nucleic acid detection directly from patient sample. All the nucleic acid is sequenced in parallel resulting in sequencing of both host and microbial reads. Syndromic approach of central nervous tissue infection, blood stream infection, respiratory, urinary tract infection and ocular panels can be done by this method. All groups of pathogen including bacteria, viruses, fungal, mycobacterial parasites (both DNA and RNA) can be detected by this method.

The advantages of this mycobacterial, parasites method are:

- Strain typing, virulence factors detection, antimicrobial resistance and host immune response can be done directly from patient sample.
- Precision medicine based approach for diagnosis

The main disadvantage is the cost as more than 90% reads will be of human origin which will be unnecessarily sequenced.

VII. NANOTECHNOLOGY[18-20]

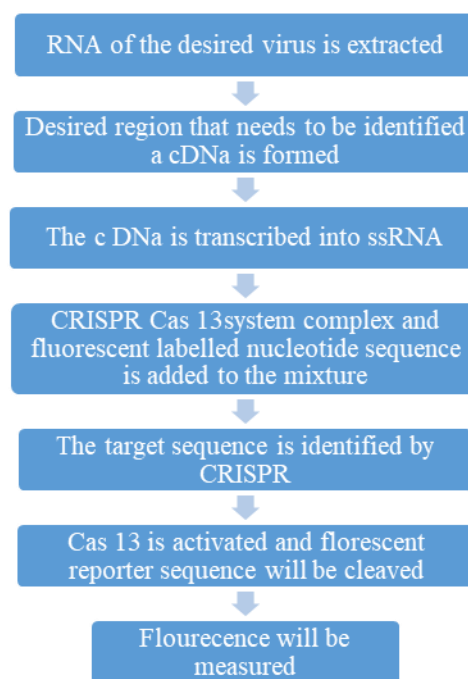
Nano means dwarf, hence it's existing or new technologies which are made miniscule sizes .Nanoparticles have been used for the following purposes

1. They are used for diagnostic and screening purpose of various infection and immune system They are available rapid, highly sensitive and specific point of care testing. Such tests are available for HIV, SARS-COV-2,Influenza to name a few. In future other infectious diseases may also be explored
2. Fluorescent nanoparticles “Quantum core crystals” have an unlimited range of sharply defined colours which might be used for fluorescent staining techniques
3. Gold nanoparticles have been used for DNA diagnostics and in DNA microarray
4. Nanotechnology has also been used in sequencing called nanopore technology
5. Nanotechnology has been sought for drug delivery system. In covid for intranasal delivery of drugs to lungs again nanoparticles have been used.
6. Their role in immune modulation have also been studied , as they can be used to decrease the cytokine release and hence prevent cytokine storm
7. Nanotechnology has revolutionized the vaccine development with application in gene therapy and tissue engineering
8. Its role in hospital infection control is worth a mention. Nanoparticles have been used in superfine filters in masks , also used in neutralization of viruses. Antipathogenic property and ability to inactivate viruses, bacteria and fungi of nanoparticles have been used in disinfection of hospital.

VIII. CRISPR IN MICROBIOLOGY[21-24]

CRISPR stands for Cluster Regularly Interspaced Short Palindromic Repeats. It is a nucleic acid detection method which is sensitive inexpensive, rapid and portable diagnostic method. The initial discovery was for detection of cDNA using Cas 9 protein. The CRISPR forms a loop shaped structure with complimentary sequence to the DNA/RNA of choice. Cas 9 and cas 12 are for DNA detection whereas Cas 13 is for RNA detection. The CRISPR technique has been tested for viral diseases like SARS-CoV-2, HIV, Hepatitis, HPV serotyping, and bacterial diseases like TB and E.coli.

Flow chart 1: Mechanism of CRISPR in diagnosis using Cas 13 protein



The CRISPR system have been in nacent stage for detection and will now be able to explore other viruses and bacterial detection within 1 hour. The drug resistance and oncogenic viruses can be detected due to various mutation using this technique.

IX. LAB ON A CHIP[25]

The lab on a chip (LOC) is one of the latest in the point of care testing and has a main advantage of lower consumption of patient sample, rapid, ease to use and has a good sensitivity and specificity. LOC method can detect DNA, RNA and protein and are used for detection of a wide variety of viruses and bacteria. LOC can be microarray devices, micro channel biodevices and micro fluidic systems. LOC are chip containing miniaturized channels, miniature pumps which are made up of basic elements of either glass, quartz or silicon. The detection in LOC is done using optical density or fluorescent based detection (Raman receptors). The conventional methods may take upto 3 days but with LOC the result is available within minutes, and can be used in remote setting in a real time mode. They have been used in detection of various bacterial diseases such as S. aureus, Vibrio, P. aeruginosa,

S. typhimurium, *E. coli*, *L. monocytogenes*, *Leigonella*, *Streptococcus*, and viruses like HIV, hepatitis B and Zika virus.

Certain newer technologies have been in pipelines to improve the LOC detection :

1. Photodiodes have been integrated with microfluidic system like ATP bioluminescence for detection of air borne pathogens
2. Smart phones can be integrated with LOC as images and data acquisition can be easier and could be decipated
3. Loop mediated isothermal amplification with LOC called as “ LAMP box” for flaviviridae family
4. Nanophotonic lab on chip platform
5. Has been tried for disease such as malaria.

X. CONCLUSION

The conventional methods are the building blocks which paved way to the current newer technologies. It has unfolded to a simple, rapid, portable, at hand detection system making the health care system accessible even to remote rural settings. This has urged the Medical researchers and Clinicians to understand these technologies for delivering a better healthcare facility. With the advancement of Automation and Digitalisation, infections can be uncovered at one's touchpoint.

REFERENCES

- [1] Digitalization, clinical microbiology and infectious diseases. A. Egli, *Clin Microbiol Infect* 2020;26:1324.
- [2] Henning C, Aygul N, Dinnetz P, Wallgren K, Ozenci V. Detailed analysis of the characteristics of sample volume in blood culture bottles. *J Clin Microbiol* 2019;57.
- [3] Bou G, Canton R, Martinez-Martinez L, Navarro D, Vila J. Fundamentals and implementation of microbiological diagnostic stewardship programs. *Enferm Infecc Microbiol Clin* 2020.
- [4] Piau A, Crissey R, Brechemier D, Balardy L, Nourhashemi F. A smartphone Chatbot application to optimize monitoring of older patients with cancer. *Int J Med Inform* 2019;128:18e23.
- [5] Glasson J, et al. Multicenter evaluation of an image analysis device (APAS): comparison between digital image and traditional plate reading using urine cultures. *Ann Lab Med* 2017;37:499e504.
- [6] Van TT, Mata K, Dien Bard J. Automated detection of *Streptococcus pyogenes* pharyngitis by use of colorex strep A CHROMagar and WASPLab artificial intelligence chromogenic detection module software. *J Clin Microbiol* 2019;57:e00811e9.
- [7] Sousa T, et al. Putative protein biomarkers of *Escherichia coli* antibiotic multiresistance identified by MALDI mass spectrometry. *Biology (Basel)* 2020;9.
- [8] IUPAC. Compendium of Chemical Terminology; McNaught, A.D., Wilkinson, A., Eds.; Blackwell Scientific Publications: Oxford, UK, 1997; Volume 2, ISBN 0-9678550-9-8.
- [9] Teengam, P.; Siangproh, W.; Tuantranont, A.; Vilaivan, T.; Chailapakul, O.; Henry, C.S. Electrochemical impedance-based DNA sensor using pyrrolidiny peptide nucleic acids for tuberculosis detection. *Anal. Chim. Acta.* 2018, 1044, 102–109.
- [10] Cui, F.; Xu, Y.; Wang, R.; Liu, H.; Chen, L.; Zhang, Q.; Mu, X. Label-free impedimetric glycan biosensor for quantitative evaluation interactions between pathogenic bacteria and mannose. *Biosens. Bioelectron.* 2018, 103, 94–98.
- [11] Narang, R.; Mohammadi, S.; Ashani, M.M.; Narang, R.; Mohammadi, S.; Ashani, M.M.; Sadabadi, H.; Hejazi, H.; Zarifi, M.H.; Sanati-Nezhad, A. Sensitive, Real-time and Non-Intrusive Detection of Concentration and Growth of Pathogenic Bacteria using Microfluidic-Microwave Ring Resonator Biosensor. *Sci. Rep.* 2018, 8, 15807.
- [12] Wu, G.; Datar, R.H.; Hansen, K.M.; Thundat, T.; Cote, R.J.; Majumdar, A. Bioassay of prostate-specific antigen (PSA) using microcantilevers. *Nat. Biotechnol.* 2001, 19, 856–860.

- [13] Xiaodong Mao, Chao Liu¹, Hua Tong, Yajun Chen³, Kangsheng Liu. Principles of digital PCR and its applications in current obstetrical and gynecological diseases. *Am J Transl Res* 2019;11(12):7209-7222
- [14] Vogelstein B, Kinzler KW. Digital PCR. *Proc. Natl Acad. Sci. USA* 96(16), 9236–9241 (1999).
- [15] Farrar JS, Wittwer CT. Extreme PCR: efficient and specific DNA amplification in 15–60 seconds. *Clin. Chem.* 61(1), 145 (2015).
- [16] Mitchell SL, Simmer PJ. Next-Generation Sequencing in Clinical Microbiology: Are We There Yet? *Clin Lab Med.* 2019 Sep;39(3):405-418.
- [17] Gaston DC, Miller HB, Fissel JA, Jacobs E, Gough E, Wu J, Klein EY, Carroll KC, Simmer PJ. Evaluation of Metagenomic and Targeted Next-Generation Sequencing Workflows for Detection of Respiratory Pathogens from Bronchoalveolar Lavage Fluid Specimens. *J ClinMicrobiol.* 2022 Jul 20;60(7):e0052622. doi: 10.1128/jcm.00526-22. Epub 2022 Jun 13.
- [18] Weiss C, Carriere M, Fusco L, Capua I, Regla-Nava JA, Pasquali M, Scott JA, Vitale F, Unal MA, Mattevi C, Bedognetti D, Merkoçi A, Tasciotti E, Yilmazer A, Gogotsi Y, Stellacci F, Delogu LG. Toward Nanotechnology-Enabled Approaches against the COVID-19 Pandemic. *ACS Nano.* 2020 Jun 23;14(6):6383-6406.
- [19] Emerich DF, Thanos CG. Nanotechnology and medicine. *Expert OpinBiolTher.* 2003 Jul;3(4):655-63
- [20] Doroudian M, O' Neill A, Mac Loughlin R, Prina-Mello A, Volkov Y, Donnelly SC. Nanotechnology in pulmonary medicine. *CurrOpinPharmacol.* 2021 Feb;56:85-92.
- [21] Sam IK, Chen YY, Ma J, Li SY, Ying RY, Li LX, Ji P, Wang SJ, Xu J, Bao YJ, Zhao GP, Zheng HJ, Wang J, Sha W, Wang Y. TB-QUICK: CRISPR-Cas12b-assisted rapid and sensitive detection of *Mycobacterium tuberculosis*. *J Infect.* 2021 Jul;83(1):54-60.
- [22] Mustafa MI, Makhawi AM. SHERLOCK and DETECTR: CRISPR-Cas Systems as Potential Rapid Diagnostic Tools for Emerging Infectious Diseases. *J ClinMicrobiol.* 2021 Feb 18;59(3)
- [23] ShihongGao D, Zhu X, Lu B. Development and application of sensitive, specific, and rapid CRISPR-Cas13-based diagnosis. *J Med Virol.* 2021 Jul;93(7):4198-4204.
- [24] Ebrahimi S, Khanbabaei H, Abbasi S, Fani M, Soltani S, Zandi M, Najafimemar Z. CRISPR-Cas System: A Promising Diagnostic Tool for Covid-19. *Avicenna J Med Biotechnol.* 2022 Jan-Mar;14(1):3-9.
- [25] Nasserri B, Soleimani N, Rabiee N, Kalbasi A, Karimi M, Hamblin MR. Point-of-care microfluidic devices for pathogen detection. *BiosensBioelectron.* 2018 Oct 15;117:112-128.