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A Comprehensive Review of Applications and Advancements in Rapid Diagnostic Technologies: Shaping the Future of Microbiological Detection

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Abstract

The present work presents a comprehensive review of the most recent developments in microbiology's fast diagnostic method. The increasing demand for quick and precise infection diagnosis has resulted in major improvements in diagnostic techniques. This study looks at the ways that more recent fast diagnostic technologies, like immunoassays, point-of-care testing (POCT) devices, and nucleic acid amplification tests (NAATs), are replacing more traditional, culture-based techniques. It outlines the technological advancements in CRISPR-based diagnostics, biosensors, and microfluidics and shows how these advances affect public health, clinical microbiology, and the management of infectious illnesses internationally. The evaluation also looks at the drawbacks and restrictions of various technologies, including manpower requirements, cost, and accessibility. Future perspectives on the incorporation of AI and ML into diagnostic systems demonstrate how these developments have the potential to transform pathogen diagnosis and enhance patient outcomes.

Keywords: advancement, diagnostic tests, CRISPR, healthcare applications, challenges.

Introduction and background

Diagnostic microbiology has been transformed by molecular biology through the use of PCR, pyrosequencing, and other technologies. The go-to lab tool for identifying different bacteria these days is PCR, although its usefulness and affordability vary depending on use (Maurer *et al*., 2011).Many deaths are attributed to infectious diseases each year, particularly in developing countries where bacteria, fungus, viruses, and parasites are the cause. For the purpose of treating patients, determining the etiological agent, and discovering novel infections, disease diagnosis is essential. Appropriate identification facilitates efficient treatment and surveillance programs to limit the spread of illness. A correct diagnosis is essential to healthcare since it establishes the course of treatment for medical professionals and helps to reduce unnecessary deaths brought on by misdiagnoses (Obande *et al*., 2020).

Rapid diagnostic testing (RDT) is promoted as a way to improve treatment outcomes by the Infectious Diseases Society of America (ASP) and the National Action Plan for Combating Antibiotic-Resistant Bacteria. Their poor performance and high implementation costs, however, are preventing their broader use. A thorough evaluation of the effects of mRDT on patients with BSIs in terms of mortality risk, time to successful therapy, and duration of stay is the goal of this systematic review (Timbrook *et al*., 2016). Sonography, digital mammography, SPECT, CT, PET, MRI, and SPECT are examples of advanced medical imaging techniques. Each has its own set of uses in medical labs and working principles (Hussain *et al*., 2022).

Types of Rapid Diagnostic Technologies:

Point-of-care tests (POCT):

50% of people worldwide suffer from infectious diseases, which provide dangers to the public's health and economy. The World Health Organization advises sensitive, cost-effective testing as well as point-of-care diagnostics since accurate diagnosis is essential (Hussain *et al*., 2019).Nanotechnology-based point-of-care diagnostics provide readily available, cost-effective, and portable instruments for the real-time diagnosis of diseases; yet, their successful use in global health necessitates quality assurance, environmental concerns, and downsizing (Thwala *et al*., 2023). POCT is a commonly used treatment by ward or outpatient personnel that is easy on patients and requires little sample preparation or prior knowledge (Choi *et al*., 2016).Point-of-care diagnostics offer instantaneous in-vitro information at crime scenes, hospitals, and ambulances. This improves public health by empowering people to self-test for stigmatized conditions and shortening the duration of treatment interventions (Park *et al*., 2022).

Lateral flow assays (e.g., pregnancy tests, COVID-19 rapid tests):

Though it has limitations such as manual operation and no automatic recording, LFIA is a widely used instrument for consumer protection, environmental monitoring, and illness detection (Omidfar *et al*., 2023).Wax printing chips, nitrocellulose membrane, cellulose paper, and colloidal gold immune labeling were utilized in a microfluidic paper-based apparatus to detect the bladder cancer biomarkers NMP22 and BTA from urine samples (Hsiao *et al*., 2021).

Lateral flow technologies for COVID-19 Detection:

Fluorescent reporters called LFIAs have been utilized to detect COVID-19 antigens in the blood of SARS-CoV-2 patients. Compared to ELISA and RT-PCR testing, they are less prevalent because of their lower sensitivity, but they offer 100% specificity and 68% sensitivity (Hsiao *et al*., 2017).

Molecular diagnostics:

PCR:

The thermostable Taq DNA polymerase known as PCR has revolutionized molecular diagnostics by generating large-scale target sequences that facilitate the rapid identification of both known and unknown alterations (Hariharan *et al*., 2021). Taq DNA polymerase, primers, and dNTPs are used in PCR to detect plant infections, particularly fungus, which changes the way disease is managed. It produces universal primers that are useful for accurately identifying fungal infections (Hariharan *et al*., 2024).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR):

Studies on the life cycle of microorganisms and virus detection are conducted using real-time polymerase chain reaction, or RT-PCR. It has an impact on techniques for amplified DNA-based mutation detection. Two-step PCR is more versatile and sensitive than single-step PCR; however, single-step PCR is less sensitive yet still effective for high throughput.

Real-Time PCR:

Fluorescence probes are used in real-time PCR to amplify DNA sequences, permitting faint results in 30-45 minutes and mitigating carry-over contamination, which is categorized by discriminating between allelic variants.

Enzymatic-Based Methods:

The initial technique for utilizing restriction enzymes to find mutations is called RFLP analysis. Other methods involve the hybridization of complementary DNA stretches at mutation sites via oligonucleotide ligation, chemical agents, and resolvase enzymes.

Electrophoretic-Based Techniques:

Genomic research is using a growing number of techniques, such as two-dimensional gene scanning, TGGE, DGGE, and SSCP, to detect mutations in genomic loci. These techniques enable high-throughput DNA screening of patients.

Solid Phase-Based Techniques/Hybridization or Blotting Techniques

Membrane-bound oligonucleotides are used in a variety of techniques, including SSCP, TGGE, DGGE, and two-dimensional gene scanning, to screen for mutations. PCR-ASO increases sensitivity by 1 ct and employs filters and DNA oligonucleotide microarrays to enable high-throughput mutation analysis (Hariharan *et al*., 2021).

Next-generation sequencing (NGS):

Nucleic acid sequencing is being revolutionized by next-generation sequencing (NGS), which is defying Moore's law by offering fast, affordable data for functional validation, variant pathogenicity assessment, accurate patient phenotyping, and genetic diagnostics (Satam *et al*., 2023).The advances in genome structure, function, and dynamics brought about by next-generation sequencing (NGS) have completely changed the field of genomics research. The utilization of NGS in gene expression, genetic variation, microbial diversity, and epigenetic modifications offers up new research avenues due to its low cost and high throughput (Buermans *et al*., 2014). Bioinformatics and wet lab skills are essential, but sequencing data delivers low noise, robustness, and quality. Unplanned sequence deletion is prevented by systems, and high sequence counts point to problems with the research. Applications of next-generation sequencing technologies include genetic variation identification, ChIP-Seq, RNA-Seq, and human gene expression investigations (Tsang *et et al*., 2021).

The usage of next-generation sequencing (NGS) technology has progressed from research to clinical settings. While long read sequencers like Oxford Nanopore technology and PacBio offer higher read accuracy, platforms like Illumina, MGI's DNBSEQ, and Ion Torrent offer cheap sequencing costs per base (Shi *et al*., 2021).

Immunoassays:

In order to overcome the difficulties associated with conventional immunoassays that need bulky equipment, researchers are investigating the combination of immunoassay with microfluidics to improve performance in POCT, increase sensitivity, and expedite procedures (Mou *et al*., 2017).The need for affordable, user-friendly point-of-care biosensors for precise disease diagnosis arose from the problems that conventional immunoassays caused patients to experience as a result of delayed findings (Ahsan *et al*., 2022).Enzyme immunoassays (EIAs) are employed in point-of-care biosensors to quickly diagnose and comprehend diseases by detecting the amounts of antigens in complicated mixtures. Methods use either direct or indirect conjugation (Murphy *et al*., *2021*).

Biosensors:

In medicine, biosensors are analytical instruments that are used to diagnose diseases and find novel treatments. They are effective in clinical settings because of their essential qualities, which include selectivity, affordability, reproducibility, sensitivity, and repeatability (Murphy *et al*., 2021).Biosensors help researchers in food safety analysis, infectious disease diagnosis, environmental monitoring, and test length reduction by precisely detecting biological molecules, improving reagent-biomarker interaction, and offering fast readout (Alsalameh *et al*., 2022). Biosensors are rapid, simple, low-cost, portable, sensitive, and quick-learning analytical tools using biological recognition molecules that require little experience to operate well (Jayamohan *et al*., 2017).

Methods

Literature Search:

We looked through PubMed, Google Scholar, Web of Science, and Scopus using the web-based search engine to find English-language systematic review articles that contrasted advancements in quick diagnostic technologies with traditional microbiology methods from inception to 2024. The search terms advancements, fast diagnosis, traditional microbiology, applications, and healthcare advantages are what we utilized. We conducted a manual search of the listed publications' references to find further pertinent research. The inclusion of unpublished papers was achieved by employing keywords to scan abstracts from 2000 to 2024.

Study Selection:

All studies assessing the development of traditional microbiology techniques as well as quick diagnostic technology were acceptable for inclusion. PCR and other commercially accessible molecular assays that yielded data were referred to as molecular diagnoses. Studies were considered included if they reported full-text articles, meta-analyses, and systematic review papers. The study was excluded if the abstract and title did not match the inclusion requirements.

Data Extraction and Analysis:

A review manager was employed to perform every meta-analysis. An extensive literature review and meta-analysis were conducted, adhering to the Preferred Reporting Items for Systematic Review Articles and Meta-Analyses (PRISMA) guidelines.

Results

167 studies that met the keyword requirements were found in the literature search. Following the elimination of duplicates, 150study titles and abstracts were examined. After removing studies that had no relevance to our search, 20 studies

were left for full text evaluation. 35 articles containing data unrelated to our meta-analysis were found during the full-text review. As a result of reviewing the references to the included research, 4 additional papers were added to the meta-analysis. Data were taken out of 64 research.

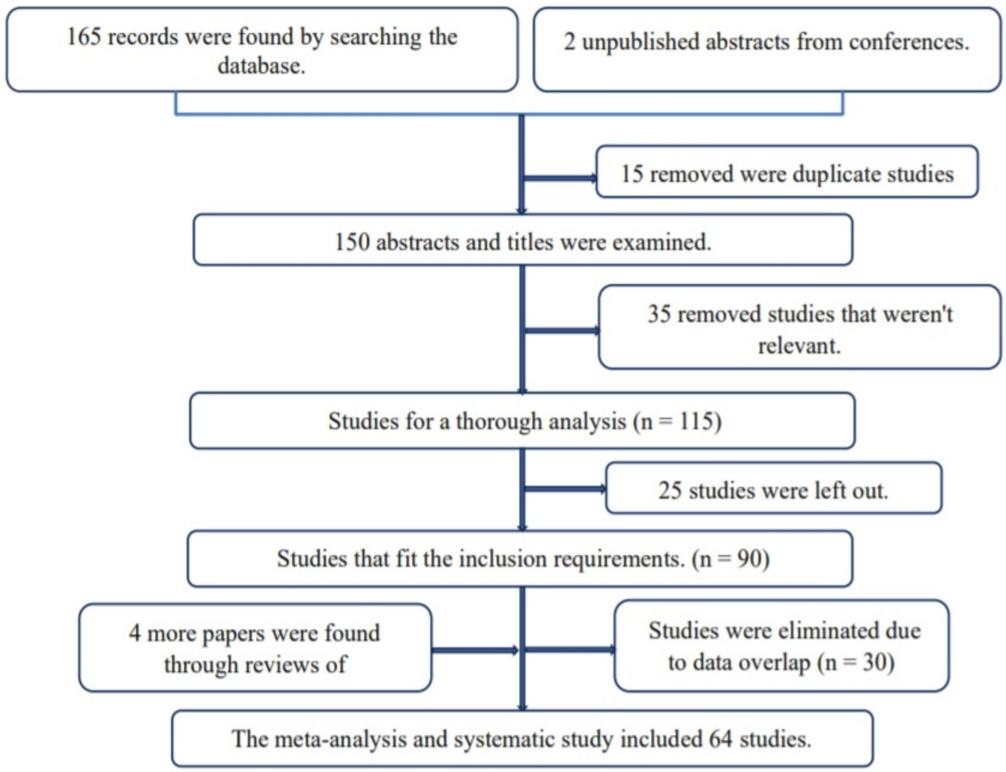

Fig. 1: PRISMA Flow Chart

Technological Advancements:

Microfluidics and lab-on-a-chip technology:

Microfluidics uses micrometer-scale channels to process low-volume fluid samples, which speeds up analysis and reaction times. Devices are becoming more affordable and smaller thanks to manufacturing advancements, particularly in the field of global health. However, scale problems and volume mismatch prevent them from being widely used (Luka *et al*., 2015). Microfluidics is a multidisciplinary branch of technology that encompasses various disciplines such as chemistry, engineering, physics, biotechnology, micro- and nanotechnology, and digital, droplet-based, and continuous flow systems (Francesko *et al*., 2019).

Microfluidic technology for drug development, drug delivery and diagnostics:

Protein expression and enzyme activity/kinetics:

Screening without cells Because they offer an in vitro perspective on a drug's effects, microfluidic devices are essential for drug research. Reagent usage is reduced via high-throughput modules that generate functional cell proteins. These tools are useful for early-stage toxicity screening and drug target research since they detect both enzyme activity and inhibition.

Diagnostics:

Research is concentrating on creating point-of-care diagnostic tools for infectious diseases like HIV. Microfluidic devices are intended to detect diseases early, automate immunoassays, and enhance sensitivity and mobility.

Microfluidic high-throughput screening:

Pharmaceutical research is increasingly using large-scale data processing and systematic screening to evaluate complex systems and interactions. Compared to traditional plate-based screening apparatus, Caliper Life Sciences' glass microchip with embedded capillaries delivers higher throughput and reduced consumption (Kumaran *et al*., 2023).

CRISPR-based diagnostics:

The gene editing technique CRISPR-Cas9 has completely changed both gene editing and molecular diagnostic testing. It enables plants to develop disease resistance, as evidenced by the unaffected tolerance to different viruses displayed by plants like *N. benthamiana* and *Arabidopsis* (Mohammad *et al.,* 2022). Highly programmable CRISPR-Cas technologies offer single nucleotide specificity and minimize false negative diagnoses. Especially in light of the COVID pandemic, they are employed in genome editing, therapy, and molecular diagnostics. High sensitivity and specificity are provided by the guide RNA that programs them (Lau *et al*., 2020), (Kanchi *et al*., 2018).

Digital and smartphone-based diagnostics:

Smartphones are transforming banking, business, entertainment, communication, and health. In China and Korea in particular, sensor applications make it possible for inexpensive, accessible health screenings and pathology exams (Rateni *et al*., 2017). Due to their affordability and ease of use, mobile diagnostics are becoming more and more popular in the environmental monitoring, agro-food, and healthcare sectors. The announcement of the Horizon Prize, which permits the use of smartphones in remote locations, represents this trend (Conte *et al*., 2024).

Advances in nanotechnology for diagnostics:

The goal of nanobiology is to decrease morbidity and death rates by developing rapid, easy, and cost-effective ways to identify clinical samples through the use of nanoscale features in clinical diagnostic software (Thwala*et al*., 2023).Through the use of nanobiosensors and biosensing platforms, medical diagnostics are improved by nanotechnology by gaining increased sensitivity, specificity, and precision. This allows for the identification of genetic abnormalities, early illness detection, and viral infection detection (Dias*et al*., 2019).

Artificial intelligence and machine learning in diagnostics

Digital health care is experiencing a revolution in disease detection and treatment because of artificial intelligence (AI) and machine learning (ML). Techniques for autonomous illness diagnosis, especially in the detection of cancer, are being developed via AI and ML. Drug development, clinical diagnostic data processing, and decision assistance are all aided by deep learning (DL), which simulates human brain activity (Iqbal *et al*., 2021), (Quazi *et al*., 2022).Artificial intelligence is able to distinguish prognostic phenotypic differences in cardiovascular illnesses and recognize congestive heart failure through the use of neural network classifiers and machine learning in echocardiography (Mishra *et al*., 2022).

Applications in Healthcare

Infectious diseases

Infectious diseases arise when pathogens infiltrate organisms, resulting in sickness and the production of toxins. The environment has an impact on the spread of viruses. These include land use, infrastructure, travel, commerce, natural disasters, climate change, and warfare (Ismoilovich *et al*., 2022). Over 1.4 million deaths and one million cases of COVID-19 had been confirmed worldwide as of November 1, 2020.

Preventing significant repercussions requires early detection. SARS-CoV-2 is the virus that infected humans more than 4,000 years ago after spreading from domesticated animals (Subramanian *et al*., 2020).

Chronic diseases:

Modern medicine and public health have increased human longevity over the past 200 years. The world's leading cause of death, chronic inflammatory illnesses, are on the rise, and hospital overcrowding due to population increase poses a threat to patient quality of life (El-Rashidy *et al*., 2021), (Nathan *et al*., 2015). A major public health concern is the diabetes epidemic, which is influenced by behavioural, social, genetic, and prenatal factors. In the world, 80 million people have type 2 diabetes, of which 28 million are affected by the condition. It causes cancer, heart disease, and other chronic issues. Among the behavioural risk factors include alcohol drinking, inactivity, poor diet, and tobacco use (Alradwan *et al*., 2024), (Pulumati *et al*., 2023).

Cancer diagnostics:

The prevalence and mortality of cancer are increasing worldwide, making it necessary to employ diagnostic probes or biomarkers for cancer detection, which will enhance prognosis (Tobore *et al*., 2020).Weight loss, lymphadenopathy, coughing up blood from the rectal area, breast lumps, and a chronic cough are all signs of cancer. A lot of patients disregard these because of problems with healthcare, cultural norms, lack of health literacy, expensive expenses, and anxiety about being diagnosed (Chaturvedi *et al*., 2019).Cancer is caused by gene abnormalities that result in uncontrollably growing cells and tumors. While current anticancer drugs have negative side effects, imaging and therapy modalities emphasize growth and division. Success depends on an early diagnosis (Eubank *et al*., 2020).

Benefits of Rapid Diagnostic Technologies:

Clinical microbiology laboratories can drastically lower morbidity, death, hospital stays, and costs by using advanced diagnostic testing technologies, such as MALDI-TOF mass spectrometry, in comparison to traditional culture processes (Olatunji *et al.,* 2024).The management of infectious diseases is limited by the use of traditional diagnostic tools such as microscopy and culture-based procedures. Portable equipment and rapid diagnostic technologies enable timely diagnosis and treatment, enhancing public health responses, decreasing the overuse of antibiotics, and enhancing disease monitoring (Ahmad *et al*., 2024).

Discussion

The study by Aliu Olalekan Olatunji et al. emphasizes the disruptive potential of portable electronics and fast diagnostic tools in healthcare while highlighting the ongoing need for research and development (Ahmad *et al.,* 2024). According to Ahmad et al., in order to improve sensitivity, specificity, and speed in AMR surveillance and microbial detection, novel approaches such as molecular techniques, mass spectrometry, biosensors, and nanotechnology are required (Vidhya *et al*., 2024). Tristan T. Timbrook *et al*. suggested mRDT as the gold standard of care since it shortened the length of therapy and decreased the risk of death in BSI patients with ASP (Timbrook *et al*., 2016). In their study, Vidhya et al. stress the value of artificial intelligence, rapid diagnostic testing, sequencing, and microbiological material quality assessment in the prevention of infectious diseases (Ivashko *et al*., 2023). M. Ivashko *et al.* draw attention to the shortcomings of the clinical diagnostic techniques used today, such as immunological, molecular biology, and omics technologies, in order to emphasize the need for quicker, more effective treatments (Oon *et al*., 2023). Oon *et al.* discovered that while digital PCR and NGS enhance the identification of aquatic harmful bacteria, practical application and large-scale use remain obstacles (Gradisteanu Pircalabioru *et al*., 2022).

Improvements in respiratory viral diagnostics, such as COVID-19-related cell culture, immunofluorescent labelling and PCR assays, were noted by Gradisteanu Pircalabioru et al. as promising for accurate, non-molecular, and molecular diagnosis (Leo *et al.*, 2020). To address interpretability issues, Leo et al. stress the necessity of reorganizing diagnostic facilities and carrying out independent validations in clinical practice 9 (Van *et al*., 2020).

Van Belkum et al. stress the significance of pre- and post-analytical optimizations, as well as FDA, EUCAST, CLSI, and public knowledge for the prompt approval and commercialization of antibiotic resistance testing (Vasala *et al*., 2020). Rapid growth-based AST technologies have limits, although Vasala et al. pointed out that they can change phenotypic AST when paired with isothermal amplification and microfluidics devices (Law *et al*., 2015). Despite their slower pace, Law et al. recommend further research be done to guarantee precise detection of foodborne infections employing sophisticated biosensor, immunological, and nucleic acid-based technologies (Fournier *et al*., 2013). Clinical Microbiology Laboratories (CMLs), when integrated with clinical environments for timely reporting and management, are revolutionizing patient care, medical decision-making, and epidemic containment, according to Fournier et al (Van *et al*., 2013). According to Van Belkum et al., in order to advance their abilities in post-culture bacterial and species identification, aspiring microbiologists must broaden their understanding in bioinformatics, communication, and data administration Paolucci (Paolucci *et al*., 2010). According to Michela Paolucci et al., blood culture alone is insufficient to identify antibiotic resistance and pathogenic organisms; blood analysis should be used in addition to culture methods to detect these traits.

Conclusion

Microbiological diagnostics has been transformed by rapid diagnostic technologies that offer accessibility, speed, and accuracy, such as molecular tests and point-of-care instruments. Precision is improved by combining CRISPR-based detection, artificial intelligence, and next-generation sequencing. Technological advancements confront difficulties in resource-constrained environments, especially when it comes to infectious diseases, and constant improvement is essential for accuracy, affordability, and user-friendliness. When combined with persistent cooperation, rapid diagnostic technologies like multiplex testing, wearable diagnostics, and real-time monitoring in microbiology have the potential to drastically alter how infectious diseases and public health are treated worldwide.

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