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Point-of-Care Diagnostic Laboratory Tests for Infectious Diseases

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Abstract

Infectious diseases pose a significant threat to global public health and the economy, with over half of the world's population at risk. Rapid, accurate, and accessible diagnostic tools are crucial for effective disease management and control. Point-of-care (POC) tests have emerged as essential solutions, particularly in resource-limited settings, offering advantages such as faster results, improved sensitivity and specificity, lower costs, and on-site detection capabilities. This review discusses the clinical needs for POC testing of major pathogens, including malaria, HIV, HPV, dengue, Ebola, Zika, and tuberculosis. It also examines key biomarkers for POC testing, such as pathogen nucleic acids, proteins, circulating microRNAs, and antibodies, and their roles in monitoring disease stages and treatment outcomes. Recent technological advancements in microfluidics and

plasmonics are highlighted as critical components of the "POCT Toolbox." Microfluidic devices enable the miniaturization and integration of laboratory diagnostic functionalities onto portable chips, while plasmonic technologies, such as surface plasmon resonance (SPR), localized surface plasmon resonance (LSPR), and surface-enhanced Raman scattering (SERS), offer high sensitivity, label-free detection, and real-time monitoring capabilities. The combination of these technologies holds promise for developing cost-effective, robust, and portable diagnostic platforms for infectious diseases. However, challenges in designing and developing these technologies remain, necessitating further research and development efforts to fully realize the potential of POC diagnostics in the fight against infectious diseases and global health crises.

Keywords: POC, Point-of-Care, Diagnostic Laboratory Tests, Infectious Diseases

Introduction

Infectious diseases, caused by pathogenic microorganisms such as viruses, bacteria, parasites, and fungi, represent a significant threat to public health and the global economy. These diseases can spread exponentially within populations in a short period, emphasizing their potential to cause widespread harm. It is estimated that over half of the world's population is at risk of contracting infectious diseases, marking them as one of the most severe challenges to humanity (H. Hwang et al., 2018).

Infectious diseases cause significant harm in developing countries, and access to effective treatment is crucial. Point-of-care (POC) tests are essential for diagnosis in areas without laboratory services. Although investment in POC tests has been limited historically, recent advancements have improved access to reliable tests for diseases like HIV, syphilis, and malaria. However, poor regulation has led to the proliferation of substandard tests, undermining trust and progress. Advocacy, training, and robust quality control systems are needed to fully realize the potential of POC testing and address the gap for other infections (Peeling & Mabey, 2010).

Point-of-care (POC) laboratories were developed to provide rapid diagnoses of infectious diseases as an alternative to centralized core laboratories. These labs operate 24/7 and deliver results within 2 hours, using techniques such as immunochromatography and real-time PCR. POC tests are often packaged into syndrome-based kits, allowing for simple sample collection, including self-sampling, and can be conducted by trained personnel without advanced biological expertise. They are particularly beneficial for remote populations, including those in developing nations and on ships, with support from core labs via modern internet connections. POC labs have proven cost-effective for diagnosing conditions like tuberculosis and sexually transmitted infections globally (Drancourt et al., 2016).

As noted, "Without diagnostics, medicine is blind" (García-Basteiro et al., 2018). Effective treatment of illnesses is contingent on accurate and timely diagnosis. Rapid, sensitive, and specific diagnostic tests are essential not only for facilitating appropriate treatment but also for controlling the transmission of infectious diseases. While central clinical laboratories offer highly sensitive and specific tests, including blood cultures, high-throughput immunoassays, polymerase chain reaction (PCR), and mass spectrometry (MS), these techniques are often labor-intensive, costly, time-consuming, and reliant on sophisticated equipment and skilled operators. In contrast, point-of-care (POC) tests offer rapid, on-site results, enabling timely and appropriate treatment, particularly in resource-limited settings. According to the World Health Organization (WHO), POC tests designed to address the challenges of infectious disease control, especially in developing nations, should adhere to the “ASSURED” criteria:

affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users.

Infectious diseases caused by pathogens pose a serious risk to public health and the economy, necessitating efficient diagnostic tools for accurate and timely detection. Point-of-care (POC) tests, which provide results near the patient, play a crucial role in identifying diseases, preventing transmission, and guiding treatment. This review discusses the clinical needs for POC testing for major pathogens like malaria, HIV, HPV, dengue, Ebola, Zika, and tuberculosis. It also compares molecular diagnostic approaches, including nucleic acid, protein, microRNA, and antibody-based methods. The review highlights recent advancements in microfluidic and plasmonic-based POC technologies (Chen et al., 2019).

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has highlighted the critical need for point-of-care (POC) diagnostics to enable timely prevention and control. Compared to traditional diagnostic methods, POC diagnostics offer advantages such as faster results, better sensitivity and specificity, lower costs, higher efficiency, and the capability for on-site detection. The development of effective POC detection methods and devices is essential for achieving these benefits. Advances in technologies such as microfluidics, micro electro-mechanical systems (MEMS), nanotechnology, and materials science have driven the creation of portable, miniaturized, cost-effective, and highly integrated POC devices for infectious disease diagnostics. This review discusses various POC detection methods, including:

- Electrochemical biosensors
- Fluorescence biosensors
- Surface-enhanced Raman scattering (SERS)-based biosensors
- Colorimetric biosensors
- Chemiluminescence biosensors
- Surface plasmon resonance (SPR)-based biosensors
- Magnetic biosensors

It also explores the development of advanced POC devices such as:

- Lab-on-a-chip (LOC) devices
- Lab-on-a-disc (LOAD) devices
- Microfluidic paper-based analytical devices (μPADs)
- Lateral flow devices
- Miniaturized PCR devices
- Isothermal nucleic acid amplification (INAA) devices

The study concludes by addressing the challenges in designing and developing these technologies and offering future perspectives. The aim is to guide the advancement of POC diagnostics for better management of infectious diseases and to support global efforts in preventing and controlling pandemics like COVID-19 (C. Wang et al., 2021).

The review begins by discussing the pathological processes and public health impacts of these microorganisms and identifying POC diagnostic needs. Key biomarkers for POC testing, such as pathogen nucleic acids, proteins, circulating microRNAs, and antibodies, are then examined for their roles in disease management. Lastly, recent innovations in microfluidics and plasmonics technologies, critical components of the “POCT Toolbox”, are reviewed. These technologies aim to enhance patient-centered diagnostic and therapeutic approaches in the fight against infectious diseases.

2. Pathogen Detection Needs at the POC

2.1 Malaria Parasites

Each year, more than 300 million individuals, primarily in tropical regions such as sub-Saharan Africa, are affected by malaria. Effective malaria case management, as outlined in the "Malaria Case Management: Operations Manual" by the WHO, depends on early diagnosis and prompt artemisinin-combined therapy (ACT) (Kim et al., 2015). The discovery of malaria

parasites in human blood was first documented in the 1880s via microscopic examination (Lee et al., 2010). Subsequently, microscopy of Giemsa-stained blood films became the gold standard for malaria diagnosis, requiring skilled operators and reliable equipment. Unfortunately, these resources are often scarce in malaria-endemic regions.

To address this gap, significant advancements have been made in the past decade to develop malaria rapid diagnostic tests (RDTs). These tests are designed to provide accurate and fast results in remote settings with limited access to clinical diagnostic resources. For instance, RDT lateral flow strips can detect malaria parasite-derived proteins in blood, producing visibly distinct lines. Additionally, Rathod et al. employed microfluidic channels to simulate capillary environments, enhancing the accuracy of in-field malaria diagnostics.

2.2 HIV

Globally, HIV affects over 40 million people, with approximately 85% residing in developing nations where diagnostic and antiretroviral therapy (ART) monitoring platforms are limited. HIV infection induces various immune system dysfunctions, primarily targeting CD4+ T-lymphocytes as host cells. The gp120 envelope glycoprotein of HIV binds to the CD4 receptor, initiating infection and subsequent cellular damage. During the early stages of HIV infection, symptoms may be absent; however, CD4+ T-cell counts decline, compromising the immune system and leaving patients vulnerable to opportunistic infections such as pneumonia. While no definitive cure exists for late-stage AIDS, antiretroviral drugs are effective in mitigating symptoms, particularly when administered during the early stages of infection.

Early detection of HIV not only improves therapeutic outcomes but also reduces the risk of transmission. Fourth-generation p24 antigen (Ag)/antibody (Ab) combination enzyme immunoassays (EIAs), which detect HIV p24 Ag and antibodies, have significantly reduced the diagnostic window to within two weeks of transmission (Stone et al., 2018). FDA-approved fourth-generation assays include ARCHITECT HIV Ag/Ab EIA (Abbott Laboratories), GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories and Walter Reed Army Institute of Research), Vitros HIV Combo Assay (Ortho Clinical Diagnostics), BioPlex 2200 HIV Ag-Ab Assay (Bio-Rad Laboratories), and ADVIA Centaur HIV Combo (Siemens Healthcare Diagnostics).

Commercially available HIV RDTs, such as the Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories), HIV 1/2/O Rapid Test Device (ABON), Determine HIV 1/2 (Alere), OraQuick Rapid HIV-1/2 Antibody Test (OraSure Technologies), and DPP HIV 1/2 (Chembio), provide rapid results and sometimes differentiate HIV-1/2 antibodies at the POC. For monitoring therapy, CD4+ T-lymphocyte enumeration and HIV viral load quantitation, which decline and rebound with infection and effective ART respectively, are critical. Conventional methods such as flow cytometry and quantitative RT-PCR, however, are limited by their long turnaround times, high costs, and reliance on sophisticated instruments and skilled operators. The WHO has emphasized the need for POC devices capable of accurate, cost-effective, and user-friendly HIV detection and monitoring in resource-limited settings. These devices must achieve a detection threshold of at least 200 CD4+ cells per μL and 400 copies of HIV per mL of blood to meet clinical requirements.

2.3 HPV

Cervical cancer, largely caused by persistent infection with oncogenic types of human papillomavirus (HPV), claims the lives of over 50,000 women annually in Africa. In the United States, prior to widespread Papanicolaou (Pap) smear screening, the incidence of cervical cancer reached 40.1 cases per 100,000 white women and 73.1 cases per 100,000 nonwhite women in selected areas between 1947 and 1948. Following the introduction of Pap screening and precancerous lesion treatments, this incidence dropped to 7.7 per 100,000 women by 2012,

highlighting the importance of early detection and screening in cervical cancer prevention (Mohammed et al., 2016).

Current gold-standard methods for cervical cancer screening in developed countries include HPV DNA testing and/or Pap cytology, followed by colposcopy and biopsy. Large trials, such as the one reported by Sankaranarayanan et al., demonstrated that a single round of HPV DNA testing in women over 30 years old could reduce advanced cervical cancer incidence and mortality by 50%. However, these tests rely on expensive laboratory infrastructure and recall systems, limiting their feasibility in resource-limited settings, where 85% of the global cervical cancer burden resides (Campos et al., 2017).

In such settings, visual inspection with acetic acid (VIA) has been recommended by the WHO as an alternative to Pap screening. Although VIA provides immediate results at a low cost, it lacks sensitivity, depends heavily on operator skill, and has no objective quality assurance measures, leading to the risk of overtreatment or undertreatment. Therefore, affordable, sensitive, and specific POC test platforms for HPV detection are urgently needed to improve cervical cancer prevention in resource-limited settings.

2.4 Dengue Virus and Ebola Virus

It is estimated that nearly 3 billion people across more than 120 countries are at risk of infection by the Dengue virus (DENV), which includes four serotypes: DENV1–DENV4 (Bhatt et al., 2013). DENV is a member of the Flavivirus genus within the Flaviviridae family, characterized by a single-stranded, positive-sense RNA genome. Transmitted via mosquitoes, DENV predominantly affects tropical and subtropical regions in Latin America and Asia. It is recognized as the most prevalent mosquito-borne viral infection and disease in humans, with an estimated 390 million new cases annually. In southern China, multiple dengue fever outbreaks have been reported in the past decade. DENV infections lead to a spectrum of conditions, ranging from dengue fever to severe and potentially fatal dengue shock syndrome (Guzman & Harris, 2015). According to the 2009 WHO revised case definitions, DENV-related diseases are categorized into three groups: 1) dengue, 2) dengue with warning signs, and 3) severe dengue (W.-K. Wang & Gubler, 2018). At present, there are no FDA-approved vaccines or specific antiviral treatments for dengue. Although the vaccine Dengvaxia is available, it is restricted to patients with prior DENV infections and is not recommended for dengue-naive individuals. Accurate and timely diagnosis of DENV is crucial to manage severe dengue cases effectively and to avoid overtreatment of conditions with similar clinical symptoms that are not caused by DENV. Existing diagnostic methods in centralized clinical laboratories include virus isolation, immunoassays for nonstructural protein 1 (NS1) antigens, reverse transcription-polymerase chain reaction (RT-PCR), and serological detection of DENV-specific IgM and IgG antibodies. Among these, RT-PCR offers the highest sensitivity and specificity, making it the gold standard for DENV detection. However, these laboratory-based approaches require costly instruments and trained personnel, limiting their application in resource-constrained regions. Simple, rapid, accurate, and affordable point-of-care tests (POCTs) for DENV detection are urgently needed to confirm suspected cases onsite promptly.

Symptoms of dengue fever are similar to those of other viral hemorrhagic fevers, including those caused by the Ebola virus (EBOV) [48]. EBOV is an enveloped virus with a nonsegmented, negative-sense, single-stranded RNA genome. First identified in 1976, EBOV has five known species: Bundibugyo, Sudan, Reston, Tai Forest, and Zaire, the last of which was responsible for over 11,000 deaths during the 2014–2016 West Africa outbreak. Diagnosis during this outbreak predominantly relied on RT-PCR assays which, while highly sensitive and specific, necessitate laboratory equipment and trained personnel, often unavailable in outbreak regions. During the 2014–2016 epidemic, less than 60% of cases were confirmed due to the limited availability of diagnostic tests. This highlights the critical need for POCT tools in Ebola outbreaks [53]. Broadhurst et al. evaluated the performance of the ReEBOV Antigen Rapid

Test kit against RT-PCR and found that the rapid diagnostic test achieved 100% sensitivity (95% CI 87.7–100). Brangel et al. developed a lateral-flow-based POCT for Sudan virus detection, integrating a smartphone application to collect results and geolocation data. This test demonstrated 100% sensitivity, and 98% specificity compared to standard ELISA. Sebba et al. created a rapid POCT employing surface-enhanced Raman spectroscopy nanoparticle tags (SERS nanotags) to differentiate Ebola from other endemic febrile illnesses, such as Lassa fever and malaria. This test required only two steps and under 30 minutes to deliver results, with 90.0% sensitivity and 97.9% specificity for Ebola (Sebba et al., 2018).

Given the highly contagious nature of pathogens like EBOV and the rapid progression of severe conditions in infected individuals, POCTs near containment facilities are also necessary in well-equipped countries. The Centers for Disease Control and Prevention (CDC) has issued guidelines on infection prevention and control during the collection, transport, testing, and disposal of samples. Real-world laboratory experiences from U.S. institutions provide practical insights into implementing POCTs within clinical workflows (Iwen et al., 2014).

2.5 Mycobacterium Tuberculosis

In 2016, approximately 10.4 million new cases of tuberculosis (TB) were reported, with less than 64% diagnosed, thereby hindering timely therapeutic interventions. Despite TB being largely treatable, it remains the leading infectious cause of death globally, accounting for approximately 1.3 million deaths annually. Achieving the goals outlined in the End TB Strategy—which targets a 90% reduction in incidence and a 95% reduction in mortality by 2035—requires enhanced TB diagnostic tools to enable timely interventions (Uplekar et al., 2015). Standard TB diagnostic methods currently include QuantiFERON-TB, liquid culture, and smear microscopy. These methods, however, often necessitate expensive equipment, specialized personnel, and large sample volumes. Accurate and rapid POCTs are vital to advancing the End TB Strategy. Significant progress has been made in TB POCT development in recent years. In December 2010, the WHO endorsed the Xpert® MTB/RIF assay for use in TB-endemic regions. This cartridge-based integrated PCR system minimizes technical expertise requirements and provides results from unprocessed sputum samples within 90 minutes. Other WHO-endorsed tools, such as the urine lateral-flow lipoarabinomannan (LF-LAM) test and the loop-mediated isothermal amplification (TB-LAMP) assay, have been developed to eliminate the need for complex instruments like thermal cyclers.

2.6 Zika Virus

Zika virus (ZIKV), a mosquito-borne flavivirus, was first identified in Brazil in 2015 and has since rapidly spread across tropical and subtropical regions in the Americas (Benelli & Mehlhorn, 2016). ZIKV is associated with congenital microcephaly, Guillain-Barré syndrome (GBS) and other severe neurological complications in newborns whose mothers were infected during pregnancy. According to an economic model by Lee et al. a ZIKV outbreak across six U.S. states could result in direct medical costs and productivity losses amounting to $0.5–2 billion. While primarily transmitted via mosquitoes, ZIKV can also spread through sexual contact, perinatal transmission, and blood transfusions. Because ZIKV-induced symptoms, such as fever and chills, resemble those of other febrile illnesses rapid and accurate detection is critical for appropriate treatment and prevention. Detection also plays a pivotal role in monitoring infection spread, managing risks during pregnancy, ensuring the safety of blood supplies, tracking vaccine efficacy, and assessing whether sexual partners carry the infection. The FDA has authorized emergency use of assays such as the Zika MAC-ELISA and the Trioplex rRT-PCR laboratory test for ZIKV detection (Mauk et al., 2017). However, these methods require centralized laboratory infrastructure, including bulky instruments and skilled

operators. The development of simple, accurate, and rapid POCTs for ZIKV is essential for effective disease management and prevention .

2.7 Biomarkers in Infectious Disease POCT

The National Institutes of Health (NIH) Director's Initiative on Biomarkers and Surrogate Endpoints defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". Biomarkers encompass nearly all molecules or cells involved in the infection process of infectious diseases, including proteins, nucleic acids, and antibodies. For instance, during HIV infection, the levels of the HIV RNA genome, capsid protein p24, and various antibodies exhibit distinct profile signatures, allowing for the assessment of different stages of the infection process, as illustrated in Fig. 2. The subsequent section delves into the roles of various biomarkers employed in POCTs for infectious diseases in monitoring disease stages and treatment outcomes.

2.8 Pathogen Nucleic Acids

Since most infectious diseases are caused by pathogens carrying nucleic acids (RNA or DNA), except for rare cases such as prions, pathogen nucleic acids are naturally suited as biomarkers for the diagnosis of these diseases. Nucleic acid tests (NATs) targeting pathogen-specific nucleic acid sequences have become extensively utilized in centralized laboratories. The quantity of pathogen genome nucleic acids correlates directly with the pathogen load during infection, such as in the quantitative detection of RNA to monitor HIV viral load during the early stages of infection and post-treatment (Gray et al., 2018). However, a significant limitation of using pathogen nucleic acids as biomarkers is the difficulty in distinguishing between infection and colonization. Moreover, conventional PCR-based NATs typically involve multiple sample purification and preparation steps, as well as costly instruments like programmable thermocyclers, making them unsuitable for point-of-care (POC) applications. Considerable efforts have been directed toward developing diagnostic tools that are accurate, simple, and cost-effective for the detection of infectious disease-specific nucleic acids. Strategies employed include replacing PCR with isothermal amplification techniques such as recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP), streamlining experimental procedures using integrated microfluidic devices, and utilizing synthetic biology approaches. Maffert et al. provided a comprehensive review of recent advancements in POC nucleic acid detection for infectious diseases.

2.9 Antibodies

The presence of pathogen-specific antibodies can serve as biomarkers to assess infection status. During the infectious process, the immune system generates large quantities of antibodies, often reaching levels significantly higher than those of the pathogen itself. These antibodies can remain elevated throughout the infection, even when antigen levels decrease substantially in later stages. For instance, during the late stage of HIV infection, anti-p24 antibodies remain detectable, whereas the p24 antigen diminishes to undetectable levels, as illustrated in Figure 2. In such cases, antibodies prove more effective for infectious disease diagnosis. From a technological perspective, immunoassays for antibody detection are generally simpler to develop compared to those targeting antigens, which necessitate the generation and preparation of specific antibodies. For example, HIV antibody tests can detect antibody levels as high as several mg/mL with notable specificity, achieving widespread success in HIV diagnosis. However, antibody tests are less effective when antibody levels do not correlate with infection stages. For instance, maternal antibodies transferred prenatally or through breastfeeding can lead to false-positive results in non-infected infants. Similarly, individuals who have not seroconverted post-HIV infection or those with no or atypical antibody responses are not suitable candidates for antibody-based diagnostic methods.

2.10 Pathogen Proteins

Pathogen proteins, such as capsid and envelope proteins, are universal components of infectious agents and serve as valuable biomarkers for diagnosing infectious diseases. For example, the HIV capsid protein p24 has long been recognized as an alternative to antibodies, which dominate the market for HIV POC diagnostics. The p24 protein is a small, high-copy-number molecule encoded by the gag gene, with a molecular weight of approximately 24 kDa. It polymerizes to form a cup-like shell that encapsulates the RNA genome of the HIV virus (Zhao et al., 2013). Like HIV RNA, p24 is present during the early stages of infection and can be detected before seroconversion. The Alere Determine™ HIV-1/2 AG/AB Combo rapid test, which simultaneously detects HIV-1/2 antibodies and HIV-1 p24 antigen, has received FDA approval and CLIA-waived status for fingerstick whole blood testing (Parker et al., 2018). However, unlike nucleic acids, which can be amplified using PCR, detecting ultra-low levels of proteins remains challenging. As a result, p24 detection lags RNA detection in the early stages of HIV infection.

2.11 Circulating MicroRNAs

MicroRNAs (miRNAs) are non-coding RNA molecules, approximately 20 nucleotides in length, that regulate gene expression post-transcriptionally. Over 60% of mammalian mRNAs are subject to miRNA regulation. During infections, miRNAs play critical roles in host immune responses, being routinely released into extracellular environments, particularly by immune cells (Robbins & Morelli, 2014), to mediate cell-to-cell communication. In 2008, circulating miRNAs were first reported in plasma and serum samples. Notably, extracellular miRNAs exhibit remarkable stability in body fluids, including plasma, serum, urine, saliva, and semen, as they are protected by RNA-binding proteins, high-density lipoprotein particles, and lipid vesicles. While the precise functions of the extracellular miRNA network remain under investigation, their potential as biomarkers for monitoring pathological states has gained substantial attention. For example, Fu et al. utilized the Exiqon miRCURY™ LNA microarray platform to detect 92 differentially expressed miRNAs in serum samples from tuberculosis (TB) patients. They observed significant upregulation of circulating miR-93* and miR-29a in TB cases compared to healthy controls. Additionally, specific plasma miRNA pairs, such as miR-495-3p combined with let-7b-5p, miR-151a-5p, or miR-744-5p, and miR-376a-3p combined with miR-16-5p, have been identified as potential biomarkers for HIV-associated neurological disorders (HAND) (Kadri et al., 2016).

2.12 Technology Advancements in Infectious Disease POCT

The past decade has witnessed significant technological progress in the development of POC tests for infectious disease diagnostics. Notable advancements include compact molecular diagnostic systems, lateral flow assays, microfluidics, plasmonic technologies, and paper-based assays. Among these, microfluidics has emerged as a promising solution, enabling the miniaturization and integration of laboratory diagnostic functionalities onto a portable chip. Concurrently, plasmonic technologies such as surface plasmon resonance (SPR), localized surface plasmon resonance (LSPR), and surface-enhanced Raman scattering (SERS) offer high sensitivity, label-free detection, and real-time monitoring capabilities, making them ideal for POC diagnostics. The combination of plasmonics and microfluidics is particularly promising for developing cost-effective, robust, and portable diagnostic platforms for infectious diseases. The following sections review recent technological advancements in microfluidics and plasmonics for infectious disease diagnostics. For detailed reviews on compact molecular diagnostic systems, which are primarily benchtop instruments.

2.13 Microfluidics

Microfluidics is a technology designed to manipulate extremely small fluid volumes (10^{-9} to 10^{-18} L), offering precise, programmable, spatial, and temporal control over fluid dynamics. This technology enables the transport, mixing, and reaction of samples and reagents within specifically designed microchambers under precise control. Key advantages include their low cost, portability, and ability to deliver rapid on-site results, making them ideal for point-of-care (POC) testing in remote areas. Innovations such as electrochemical assays, smartphone integration, and 3D fluidic designs enhance their accuracy and multiplexing capabilities.

Paper-based diagnostics offer a promising solution for improving healthcare access in underdeveloped regions, though further standardization and technological integration are needed to maximize their impact (Syedmoradi & Gomez, 2017). As such, microfluidics is an ideal platform for developing POC tests due to its desirable features, including automation, integration, and miniaturization. The subsequent sections highlight recent advancements in the application of microfluidic technology for infectious disease POC testing.

During malaria infection, infected red blood cells (iRBCs) exhibit progressive loss of deformability as the parasites mature within the cells. Leveraging this phenomenon, Hou et al. designed and developed a microfluidic device to explore the feasibility of using deformability as a biomarker to monitor the stages of malaria infection. Their findings indicated that iRBCs with reduced deformability were more likely to be displaced toward the walls of microfluidic channels. By segmenting the primary microchannel into smaller side channels, they achieved isolation of over 80% of iRBCs at the trophozoite/schizont stages within these side channels. However, since other conditions like sickle cell anemia also affect RBC deformability, the assay's specificity requires further enhancement. As illustrated in Fig. 3C, Wang et al. introduced an automated microfluidic device capable of detecting single-base variations in multi-drug-resistant M. tuberculosis. This device integrated several functionalities, including cell lysis, DNA isolation, PCR amplification, and signal readout, into a compact cartridge (H. Wang et al., 2012). Micropillar arrays within the microchannels were employed to enhance the interaction surface for DNA adsorption, thereby improving the sensitivity of colorimetric signal readout.

The Sia research group at Columbia University developed a point-of-care (POC) microfluidic chip for simultaneous detection of HIV and syphilis, utilizing silver-enhanced immunoassays. Their design incorporated air bubbles to separate reagents within the microfluidic channels and silver reduction to amplify colorimetric signals, achieving ELISA-equivalent sensitivity and specificity in under 20 minutes. Subsequently, they integrated this microfluidic chip into a small cartridge readable by mobile devices, such as an iPod Touch. Watkins et al. introduced a microfluidic chip for counting CD4+ and CD8+ T cells to monitor HIV infection. The chip utilized differential electrical impedance measurements. Their system detected specific spikes in impedance amplitude and width as CD4+ or CD8+ lymphocytes traversed designated regions within the microfluidic channel. The method enabled lymphocyte counting within 20 minutes, providing results comparable to flow cytometry. Similarly, Lee et al. devised an integrated microfluidic platform for diagnosing dengue virus (DENV) infection by detecting specific IgG and IgM antibodies. This system utilized magnetic microbeads and micromixers to capture target antibodies efficiently. The on-chip magnetic coils facilitated the collection of purified antibodies for subsequent fluorescence detection.

Paper-based microfluidics, known for its affordability and user-friendly design, has proven valuable for POC testing in resource-constrained environments (Magro et al., 2017). The technology's compatibility with colorimetric readouts further enhances its utility in such settings. Whiteside et al. developed a paper-based microfluidic analytical device (μPAD) for detecting antibodies to the HIV-1 envelope antigen gp41. Their design required minimal sample

volumes (1–10 µl) and provided results within an hour. Additional innovations employing microfluidics and paper-based systems for infectious disease detection have also been reported.

2.14 Plasmonic Technologies

Plasmonics investigates the interactions between light and conductive electrons within metallic nanomaterials. Common plasmonic materials include gold, silver, and aluminum. These materials have been engineered to support POC applications due to their label-free nature, high optical tunability, and sensitivity to changes in their surrounding environment. For instance, Peng et al. developed a coulometric POC assay utilizing phage-induced gold nanoparticle aggregation to detect bacterial pathogens. Recent advancements in sensitive optical transducers have further accelerated plasmonic technology development. Among optical sensing methods, the unique surface plasmon resonance (SPR) properties of plasmonic materials make them particularly promising for clinical diagnostics. SPR-based sensors exploit the sensitivity of plasmonic materials to changes in the dielectric properties of their environment, alongside electromagnetic field enhancement near noble metal nanostructures. Two major classes of plasmonic sensors have emerged: localized surface plasmon resonance (LSPR) and surface-enhanced Raman scattering (SERS) sensors.

LSPR sensors leverage the extreme sensitivity of plasmonic nanomaterials to refractive index variations. For example, they have been utilized to achieve label-free, fluorescence-free, and repeatable HIV viral load detection from unprocessed whole blood. This sensing platform detects biomarker binding events that induce LSPR wavelength shifts, allowing for sensitive, specific, and rapid detection of various HIV subtypes within an hour. Additionally, a prism coupling configuration has been employed for label-free protein detection. In this approach, sample capture causes a refractive index change, which alters reflected light intensity. Paper-based plasmonic devices, offering benefits such as high surface area, portability, and cost-effectiveness, have been utilized for selective and sensitive protein biomarker detection. These properties make plasmonic paper devices ideal for POC diagnostics in low-resource settings.

SERS sensors rely on the significant amplification of Raman scattering signals from analytes adsorbed on nanostructured metallic surfaces. Efforts to design and fabricate SERS-based devices have focused on achieving strong signal enhancement and reproducibility. A SERS-based lateral flow assay for staphylococcal enterotoxin B detection has demonstrated ultrahigh sensitivity compared to ELISA-based methods (J. Hwang et al., 2016).

Conclusion

The advent of point-of-care (POC) diagnostics has revolutionized the field of infectious disease management, offering rapid, cost-effective, and accessible diagnostic solutions. These tests are particularly vital in resource-limited settings where centralized laboratories may be unavailable, enabling timely and precise identification of infectious diseases such as malaria, HIV, HPV, tuberculosis, dengue, Ebola, and Zika virus. By leveraging biomarkers like nucleic acids, proteins, circulating microRNAs, and antibodies, POC diagnostics have demonstrated the potential to monitor disease stages and guide effective treatment strategies.

Technological advancements in microfluidics and plasmonics have further bolstered the capabilities of POC platforms, providing enhanced sensitivity, specificity, and portability. Microfluidics allows the integration of complex laboratory functions into compact devices, facilitating automation, miniaturization, and sample-to-answer workflows. Simultaneously, plasmonic technologies such as surface plasmon resonance (SPR), localized surface plasmon resonance (LSPR), and surface-enhanced Raman scattering (SERS) have introduced highly sensitive and label-free detection mechanisms that are invaluable in clinical and field settings.

Despite these advancements, several challenges remain, including the need for improved specificity, better regulatory frameworks, and robust quality control systems to ensure the reliability and scalability of POC diagnostics. Continued research and interdisciplinary collaboration will be critical to overcoming these barriers, further refining POC technologies, and extending their reach to underserved populations.

In conclusion, POC diagnostics represent a transformative approach to addressing the global burden of infectious diseases. Through innovative integration of biomarker research and cutting-edge technologies, these platforms have the potential to significantly improve public health outcomes, reduce healthcare disparities, and contribute to the global fight against infectious diseases.

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