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Low-Cost Microfluidic Systems for Detection of Neglected Tropical Diseases

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Abstract

Neglected tropical diseases (NTDs) affect tropical and subtropical countries and are caused by viruses, bacteria, protozoa, and helminths. These kinds of diseases spread quickly due to the tropical climate and limited access to clean water, sanitation, and health care, which make exposed people more vulnerable. NTDs are reported to be difficult and inefficient to diagnose. As mentioned, most NTDs occur in countries that are socially vulnerable, and the lack of resources and access to modern laboratories and equipment intensify the difficulty of diagnosis and treatment, leading to an increase in the mortality rate. Portable and low-cost microfluidic systems have been widely applied for clinical diagnosis, offering a promising alternative that can meet the needs for fast, affordable, and reliable diagnostic tests in developing countries. This review provides a critical overview of microfluidic devices that have been reported in the literature for the detection of the most common NTDs over the past 5 years.

1. INTRODUCTION

Neglected tropical diseases (NTDs) are a group of diseases that affects especially marginalized populations in tropical and subtropical regions that do not have the support of government agencies in terms of health care. Thus, these regions suffer from a lack of investment in hospital infrastructure, clinical laboratories, and medicines. The term NTD emerged approximately 20 years ago and is directly related to diseases that occur in areas of poverty (1, 2). Despite two decades of considerable advances in health care and control of NTDs, they continue to be responsible for the deaths of thousands of people and thus remain a public health problem. Therefore, early diagnosis of NTDs is crucial to control and prevent the spread of pathogens to people (3, 4).

Portable diagnostic devices have emerged as a revolutionary tool for clinical diagnosis over the past decade. These tools can now provide fast and accurate answers in a matter of minutes and be used for point-of-care (POC) analysis. The need for POC testing is critical because, currently, several diseases have been reported globally, but they occur mainly in endemic countries (5, 6). As medical resources are limited in these regions, the demand for POC diagnosis has increased exponentially, especially after the recent COVID-19 outbreak (7, 8).

Regarding the development of POC diagnostic devices, the World Health Organization (WHO) has proposed specific criteria that must be followed (**https://www.who.int/**). According to the WHO, the devices must be affordable, sensitive, specific, user-friendly, rapid and robust, equipment free, and deliverable to end users (ASSURED) (9). Microfluidics is an important tool for POC tests because it is associated with the handling and control of fluids in micrometer-sized channels (10). It has several advantages for on-site diagnosis including reduced consumption of reagents and samples, low waste generation, short analysis time, and the possibility to integrate multiple analytical procedures in a single platform offering sample-in-answer-out capabilities (10–16). Many substrate materials are available for manufacturing microfluidic platforms. Conventional materials like glass, quartz, and silicon were initially explored for this purpose using standard photolithography. However, this is a laborious process and requires equipment and cleanroom facilities that are available to a limited number of research groups (17–19). Alternative materials based on elastomeric, flexible, or thermoplastic polymers were then proposed for the development of microfluidic devices through rapid prototyping protocols, enabling their production at a large scale (20–23). More recently, polyester-toner and paper-based substrates have emerged as almost zero-cost materials for the development of disposable devices for multipurpose applications (24–30). Microfluidic devices fabricated from most of the mentioned materials match the WHO criteria for diagnostic devices.

Therefore, the development of low-cost miniaturized devices for diagnostics is being widely explored for use in POC diagnostics. These devices are usually classified according to the principle of the assay used in the test. It can be an immunoassay, which detects an antigen or antibody generated in response to an infection; a nucleic acid-based assay that detects through the pathogen's genetic material; or a biomarker-based device that is based on the measurement of the concentration of biomarkers (3, 31–33). From this perspective, this review covers the state-of-the-art developments in low-cost microfluidic systems for detecting NTDs over the past five years.

2. NEGLECTED TROPICAL DISEASES

Knowing the symptoms and causes of diseases helps in early detection, which allows effective treatment and continuous monitoring of the diseases. NTDs can be classified into protozoal, viral, parasitic, helminthic, and bacterial diseases. This review discusses examples of microfluidic devices used to diagnose the most common NTDs such as malaria, leishmaniasis, Chagas, human

African trypanosomiasis, dengue, Zika, chikungunya, Ebola, rabies, ascariasis, lymphatic filariasis, and leprosy.

2.1. Protozoal Diseases

Protozoa are unicellular eukaryotes that can cause life-threatening infections, depending on the species and host immunity. The most common NTDs caused by these species include malaria, leishmaniasis, Chagas, and African human trypanosomiasis. Among these, malaria is the protozoal disease with the highest number of cases and deaths, estimated to be ca. 247 million and 619 thousand, respectively, worldwide in 2021 (34). Therefore, an overview of protozoal diseases diagnostic methods is presented.

2.1.1. Malaria. Malaria is an infectious disease transmitted by mosquitoes infected with parasitic protozoans called *Plasmodium*. Malaria affects billions of people, usually in tropical and subtropical countries, and typical symptoms include fever, abdominal discomfort, fatigue, chills, headaches, and vomiting. When untreated, malaria can be severe and lethal (35–39).

Yang et al. (40) reported the development of a high-throughput and label-free microfluidic cell deformability sensor for quantitative parasitemia measurement determination for *Plasmodium falciparum*–infected red blood cells. The measurement of cell deformability was performed by evaluating the transit time as each red blood cell squeezes through a microscale constriction. The microfluidic sensor was able to differentiate malaria stages, allowing rapid and low-cost malaria diagnosis in remote regions where resources and equipment are limited.

An immunoassay using magnetic beads and quantum dots for the capture and detection of histidine-rich protein 2 (the most common biomarker for malaria) in an automated droplet-based microfluidic device was described by Kim et al. (41). This methodology allowed quantitative and sensitive measurement of the antigen with an overall assay time of 1.5 h.

Rackus et al. (42) developed a fully integrated digital microfluidic preconcentration technique called "preconcentration by liquid intake by paper" and applied it to an on-chip immunoassay for *P. falciparum* L-lactate dehydrogenase (PfLDH). This preconcentration technique made it possible to isolate analytes present in large volume samples and then resuspend them into smaller volumes for further processing and analysis, demonstrating its use for a wide range of applications.

A multiplexed microfluidic loop-mediated isothermal amplification (LAMP) array for rapid and low-cost detection of malaria-related parasites was reported by Mao et al. (43). The system was tested for four *Anopheles* and two *Plasmodium* species and was demonstrated to be a high-throughput, sensitive, and specific technique.

Fraser et al. (44) described a portable microfluidic aptamer-tethered enzyme capture biosensor. In the reported biosensor, the aptamers were coated onto magnetic microbeads for magnet-guided capture, washing, and detection of the biomarker. To do so, the authors incorporated three microfluidic chambers in the device, which enabled magnet-guided equipment-free colorimetric detection of the *P. falciparum* lactate dehydrogenase enzyme.

Choi et al. (45) reported a film-based immunochromatographic microfluidic device for *P. falciparum* lactate dehydrogenase and *Plasmodium vivax* detection. The microfluidic channel was patterned on a poly(ethylene terephthalate) adhesive and assembled with polycarbonate film that was activated with oxygen plasma for modification of the surface with an avidin-biotin linker for antibody immobilization. Fluorescent detection was performed using an enzyme-linked immunosorbent assay (ELISA).

Capillary-driven microfluidic chips were developed for malaria antigen *P. falciparum* histidine-rich protein 2 detection (46). This system was based on the combination of fluorescence assay with capture antibody functionalized beads, self-assembled in a capillary-driven microfluidic chip

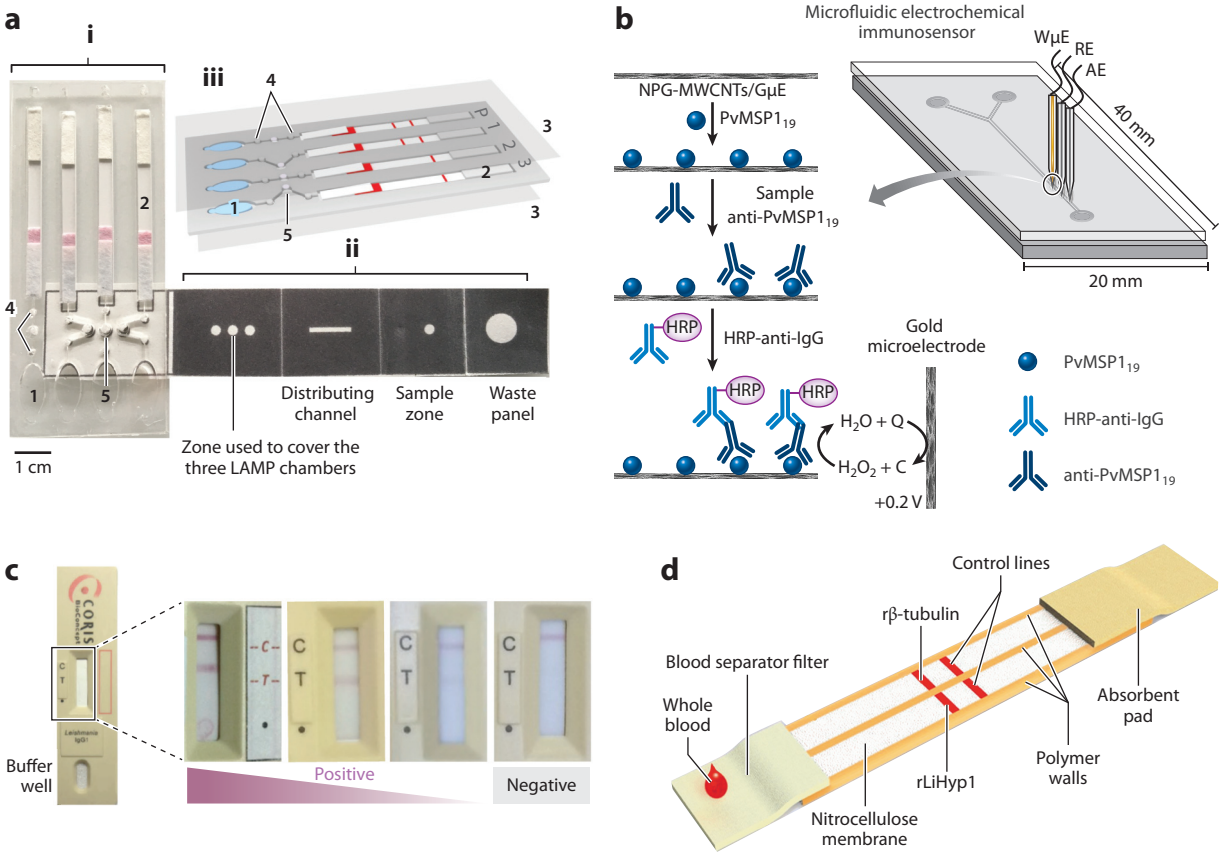

Figure 1

(*a*) The paper-based microfluidic detection device enables detection of malaria. The system is composed of a (*i*) paper-plastic lateral flow diagnostic device with an (*ii*) attached foldable paper strip. (*iii*) Schematic of the plastic microfluidic device and the numbers highlight its key features (1, buffer chambers; 2, detection strip; 3, acetate films; 4, filter paper–based valves; and 5, filter paper for LAMP reaction). Panel adapted from Reference 47 (CC BY 4.0). (*b*) Portable microfluidic electrochemical immunosensor for *Plasmodium vivax* antibody detection in human serum samples. Panel adapted from Reference 53; copyright 2021 Elsevier. (*c*) Rapid diagnostic test for monitoring IgG1 levels in visceral leishmaniasis patients. Panel adapted from Reference 59 (CC BY 4.0). (*d*) Rapid diagnostic test for human visceral leishmaniasis in a laser direct-write lateral flow device. Panel adapted from Reference 55 (CC BY 4.0). Abbreviations: AE, auxiliary electrode; HRP, horseradish peroxidase; IgG1, immunoglobulin G1; LAMP, loop-mediated isothermal amplification; NPG-MWCNT/GμE, nanoporous gold-multiwalled carbon nanotube/gold microelectrode; PvMSP1$_{19}$, *P. vivax* merozoite surface protein 1; RE, reference electrode; WμE, working microelectrode.

and an electroless silver staining technique. The analysis was successfully applied to spiked human serum samples within 20 min using a few microliters of sample and reagents.

A microfluidic paper-based analytical device (μPAD) was developed using origami to perform multiplexed DNA-based diagnostics for malaria (47). The proposed μPAD was based on vertical flow sample processing, paper folding for whole-blood sample preparation, isothermal amplification, and a lateral flow detection with simple visualization system (**Figure 1*a*, subpanels *i–iii***). With this device, the authors demonstrated the detection of 98% of individuals infected with malaria in the first double-blind study, which involved collecting whole-blood fingerstick samples from students in rural Ugandan schools.

Malpartida-Cardenas et al. (48) developed a fully electronic and homemade lab-on-chip platform for quantitative *P. falciparum* malaria diagnosis and identification of mutations related to

drug resistance. The lab-on-chip platform was composed of a motherboard printed circuit board used for data readout, a cartridge printed circuit board to host the microchip and the microfluidic chamber, a microchip including an array of sensors, and an external thermal controller. This non-optical DNA sensing method was performed using ion-sensitive field-effect transistors fabricated using unmodified complementary metal-oxide-semiconductor (CMOS) technology and coupled with LAMP, making it possible to avoid a complex sample preparation step.

A microchannel capillary flow assay chemiluminescence-based ELISA with lyophilized chemiluminescent reagents was developed for POC detection of histidine-rich protein 2 using a smartphone (49). The microchannels allowed the sequential flow of immunoassay reagents to a chemiluminescence reaction area with a single sample loading step. A sandwich ELISA was successfully applied to *P. falciparum* histidine-rich protein 2 analysis with a limit of detection (LOD) of 8 ng mL^{-1}.

Ruiz-Vega et al. (50) described an electrochemical device for POC analysis and quantification of *P. falciparum* lactate dehydrogenase in blood samples. This system was produced with a double-sided screen printing, a magnet, a whole-blood filtration unit, and absorbent pads that allow a single-step immunoassay. The detection of *P. falciparum* lactate dehydrogenase was performed in <20 min.

Lee and collaborators (51) reported a platform based on microfluidic microplate-based immunoassay for the detection of *P. falciparum* lactate dehydrogenase in <90 min. This platform used a small amount of reagents (5 μL), and it was possible to analyze *P. falciparum* lactate dehydrogenase in human serum in samples containing concentrations up to 1 pg μL^{-1}.

A protocol to compare a μPAD-based test using nucleic acid amplification with the routinely used lateral flow immunochromatography-based test for malaria diagnosis was developed by Adiga et al. (52). This protocol used statistical analysis to compare cost-effectiveness. Regiart et al. (53) recently described a portable microfluidic electrochemical immunosensor for *P. vivax* antibody detection in human serum samples. The platform was composed of a nanostructured gold surface containing multiwalled carbon nanotubes and *P. vivax* merozoite surface protein 1 ($PvMSP1_{19}$) immobilization (**Figure 1*b***). The proposed method was compared to ELISA and presented shorter analysis time, lower LODs and relative standard deviation values, and lower reagent consumption, and it was successfully applied in human serum samples for POC diagnosis.

2.1.2. Leishmaniasis. Leishmaniasis is caused by a protozoan of the genus *Leishmania* and transmitted by the bite of infected female phlebotomine sandflies. The symptoms are usually weight loss, fever, anemia, and bleeding and can lead to death if not treated. The most common forms are visceral leishmaniasis, also known as kala-azar, and cutaneous leishmaniasis. Visceral leishmaniasis is the most severe variation of leishmaniasis and is caused by the species *Leishmania donovani* and *Leishmania infantum* (54–58).

Ferreira et al. (54) developed a disposable surface plasmon resonance biochip to detect *Leishmania* spp. This immunosensor was produced using mono- and polychromatic sources immobilized with synthetic peptide LC2 derived from a mimotope of *Leishmania chagasi* antigen, and it was possible to obtain fast and real-time confirmation of the negative and positive patients.

Marlais and collaborators (59) demonstrated a rapid diagnostic test and ELISA assay for monitoring immunoglobulin G1 (IgG1) levels in visceral leishmaniasis patients. A positive result for visceral leishmaniasis using this test could also be used to diagnose post kala-azar dermal leishmaniasis. The microfluidic system consisted of a cassette with a nitrocellulose membrane, a sample pad, a conjugated pad, and an absorbent pad backed with a plastic strip. Detection could be accomplished with the naked eye (**Figure 1*c***).

In 2019, a rapid diagnostic test was developed for visceral leishmaniasis using a laser direct-write lateral flow device with double-channel geometry on a low-cost paper platform (55). The

system consisted of a laser-patterned microfluidic device and two recombinant *Leishmania* proteins, rβ-tubulin and rLiHyp1 (**Figure 1*d***). The test was more sensitive and specific than one other currently available commercial diagnostic assay and allowed for POC analysis for a large number of patients.

2.1.3. Chagas. Chagas is a disease caused by the protozoan parasite *Trypanosoma cruzi*, which is a vector-borne triatomine bug–transmitted disease that principally affects the endemic areas of Latin American countries. Chagas can be transmitted by blood, congenitally during pregnancy, during organ transplantation, and in laboratory accidents. The treatment for this disease is minimally effective for most chronic patients and its diagnosis is difficult because of the lack of available tools (60–63). For these reasons, the development of new methods and tools is necessary to make diagnosis of Chagas disease rapid, easy, and inexpensive.

Janissen et al. (64) developed an indium phosphide nanowire biosensor through the functionalization and immobilization of biomarkers on the surface using ethanolamine and poly(ethylene glycol) for Chagas detection (**Figure 2*a***, **subpanels *i–iv***). The device was fabricated by nanowire alignment through poly(dimethylsiloxane) (PDMS) microfluidic channels (100 μm wide and 50 μm deep) over electrode arrays. The PDMS microfluidic device was designed to contain 4 different channels with 16 individual electrodes per channel. The authors used the device to test for a specific antigen for Chagas. Results showed that the nanowire biosensor performance exceeded others made of materials such as graphene, graphene oxide, and MoS_2 for DNA and protein analysis. The calculated LOD was as low as 6.0 fmol L^{-1}, showing that the biosensor was highly sensitive and able to detect low concentrations of the Chagas disease biomarker (IBMP8-1) and could be used as a robust and reliable alternative tool for diagnosing chronic infections.

2.1.4. Human African trypanosomiasis. Human African trypanosomiasis is a vector-borne parasitic infection caused by a protozoan from the genus *Trypanosoma*. The transmission to humans is through bites by infected tsetse flies, which can be infected from humans or animals harboring human pathogenic parasites. The disease has two stages, and the symptoms can vary according to the infected tissue. In general, they include fever, headaches, and joint pain (arthralgia); in the worst scenario, it can cause confusion, change in behavior, coordination issues, and sleeping cycle disturbance due to central nervous system infection. Therefore, to avoid risky procedures in treatment, an early diagnosis is recommended. The diagnosis and treatment are complex and often require skilled technicians (9, 65–67). Because human African trypanosomiasis is a worrisome disease, the development of analytical tools to facilitate the process of its diagnosis is important.

Voyton et al. (68) described the use of a microfluidic system (CellASIC ONIX2) to monitor living trypanosomes. The authors reported a change in the intracellular glucose in *Trypanosoma brucei* cells when the extracellular glucose concentration was altered. Because glucose transport and metabolism are vital for *T. brucei*, the study was important for understanding its homeostasis mechanism and showed advances in the single-cell analysis of African trypanosome procyclic form (PCF) and bloodstream form (BSF).

Wan et al. (69) reported a handheld digital microfluidics (DMF) device for DNA detection via LAMP, called LampPort. In this system, the droplets and temperature manipulation were performed in real time and controlled by hardware using Bluetooth. The device was fabricated to contain a bottom plate with an array of chromium electrodes covered with SU-8-negative photoresist and a top plate of indium tin oxide (ITO)-coated glass. Both the bottom and top plates were also coated with Teflon, creating a hydrophobic surface for droplet transportation, all in a sandwiched structure sealed with ultraviolet (UV) glue. It was used to detect a unicellular protozoan, *T. brucei* with the naked eye (**Figure 2*b***).

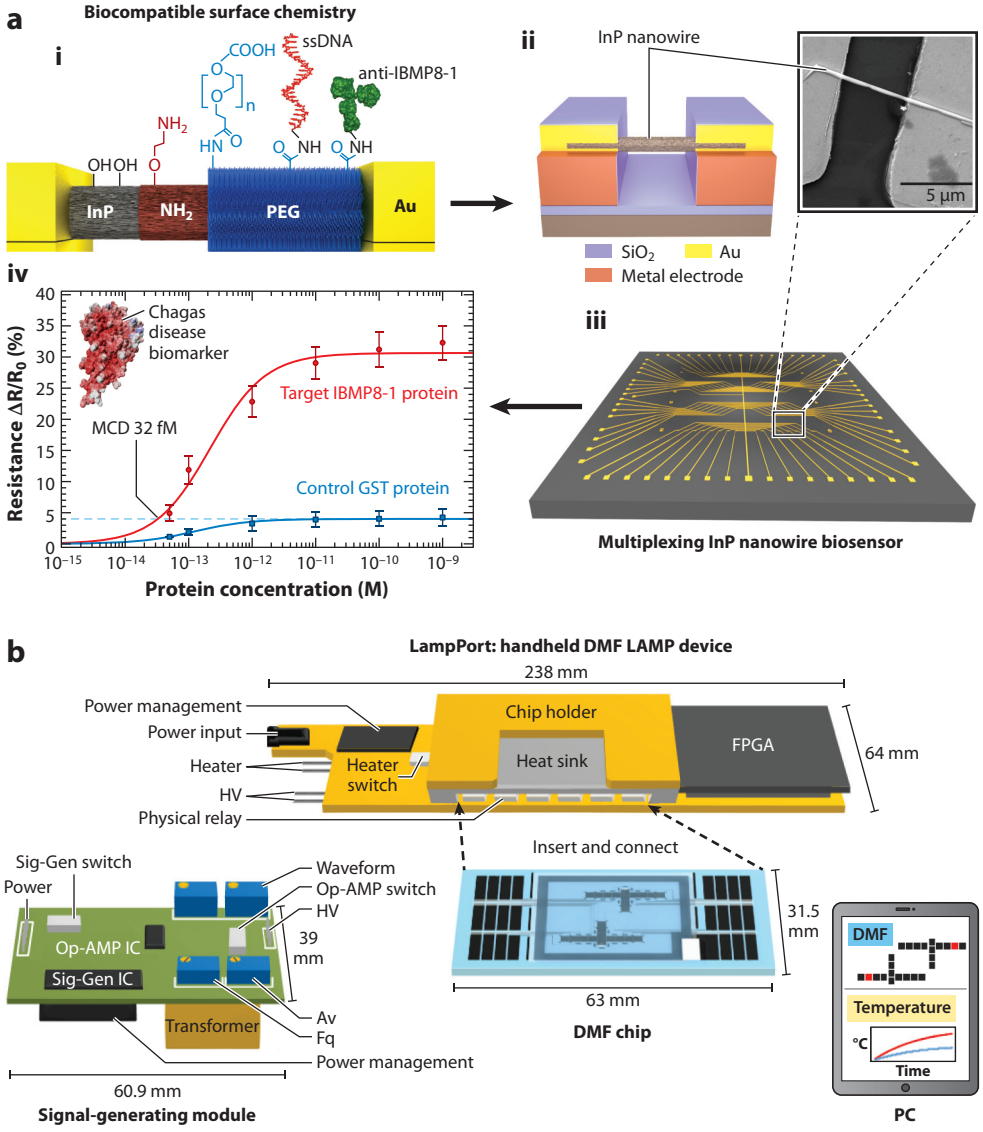

Figure 2

(*a*) Biosensor device based on InP nanowires for Chagas disease detection. (*i*) A surface functionalization and covalent immobilization of biomarkers was performed and then (*ii*) applied to InP nanowires of the (*iii*) multiplexing InP nanowire biosensor to obtain (*iv*) electrical measurement with titration. Panel adapted with permission from Reference 64; copyright 2017 American Chemical Society. (*b*) Handheld digital microfluidic device to detect human African trypanosomiasis. Panel adapted with permission from Reference 69; copyright 2019 Springer Nature. Abbreviations: Av, voltage amplification; DMF, digital microfluidic; FPGA, field-programmable gate array; Fq, frequency range; HV, high-voltage; LAMP, loop-mediated isothermal amplification; InP, indium phosphide; MCD, minimal specific detection concentration; Op-AMP, operational amplifier; PEG, poly(ethylene glycol); R, resistance; Sig-Gen, signal generating; ssDNA, single-stranded DNA.

2.2. Viral Diseases

Among the NTDs caused by viruses, dengue, Zika, chikungunya, Ebola, and rabies are the most common. The first three viruses are transmitted by infected *Aedes aegypti* and *Aedes albopictus*

mosquitoes that live in tropical and subtropical areas, while Ebola and rabies are transmitted through contact with infected body fluids. Most cases of human rabies are obtained by a dog's bite, but canine vaccination can help prevent rabies (57, 70–72).

2.2.1. Dengue, Zika, and Chikungunya. Dengue is caused by the dengue virus (DENV), which has four different serotypes that are transmissible: DENV1, DENV2, DENV3, and DENV4 (73, 74; **https://www.ecdc.europa.eu/en**). Zika virus (ZIKV) was discovered in 1947 in Uganda, and its most significant outbreaks occurred in 2013 when 30,000 individuals were infected in French Polynesia. In 2015–2016, 40,000 pregnant women were infected in South America, resulting in 4,000 cases of ZIKV-associated microcephaly in Brazil (73). The chikungunya virus (CHIKV) causes chikungunya fever, which, despite having affected the entire world, is endemic in Africa, South and Southeast Asia, and South and Central America. The main symptoms are similar to those of DENV, ZIKV, and CHIKV, such as fever, headache, myalgia, joint swelling, rash, and arthralgia (73, 75–77).

Several studies have described the development and application of microfluidic devices for detecting viruses with good precision and accuracy. Iswardy et al. (78) proposed a bead-based and antibody-based immunofluorescence assay in a microfluidic dielectrophoresis platform to detect DENV in vitro. The microfluidic platform was manufactured on an Au/Ti/glass sheet using photolithography. Although the fabrication process may be considered laborious, these devices provided attractive advantages regarding detection time (5 min), low sample consumption (15 μL), and a LOD of 10^4 PFU mL^{-1}. In addition, the ability to reuse the microfluidic chip more than 50 times may also be considered an outstanding feature.

Yuzon et al. (79) described the detection of dengue nonstructural protein 1 (NS1) in individuals through a microfluidic hybrid chip fabricated from cellulosic and poly(methylmethacrylate) (PMMA) substrate with a lateral flow immunoassay. The chip proved to be a promising tool for diagnosing DENV infection because it presented an LOD of 84.7 ng mL^{-1}; the concentration of the protein target found in infected patients is 50 μg mL^{-1}. The device offered interesting advantages including a short response time (2 min), low sample volume requirements, and practical design, all of which enable its use in POC settings.

Mendes et al. (80) reported a miniaturized disposable polyester-toner device for molecular diagnosis. This uses the reverse transcription loop-mediated isothermal amplification (RT-LAMP) technique with only one step to detect the DENV4 target in real serum samples. The authors used a simple heating block to control the reaction temperature for 15 min at 72°C and later added a SYBR Green I (SG-I) DNA intercalator. This enabled visual detection on the chip within 2 min with the aid of a UV source with a wavelength of 320 nm. The observation of bright green fluorescence indicated a patient infected with dengue (**Figure 3*a***). The device exhibited satisfactory analytical performance, with an LOD of 0.8 fg μL^{-1} for RNA.

Yuan et al. (81) exploited fluorescence detection to detect dengue biomarkers. The authors built a multiplexed imaging optical system with an infinite correction microscope device to image beads in the microfluidic channel and used portable microfluidic chips acquired from Microfluidics ChipShop. They applied this to the simultaneous detection of NS1 protein and specific IgGs of DENV using microspheres. A correlation between the fluorescence intensity and biomarker concentration was established to quantitatively analyze biological fluid samples. The system presented low limits of quantification for NS1 (7.8 ng mL^{-1}) and IgG (15.6 ng mL^{-1}).

μPADs for the detection of DENV NS1 protein in buffer, cell culture media, and human serum were developed using a sandwich immunoassay by Prabowo et al. (82). The paper surface was functionalized with anti-dengue NS1 monoclonal antibodies. The performance of the device was tested with the naked eye, a scanner, and a smartphone camera, allowing the authors to reach

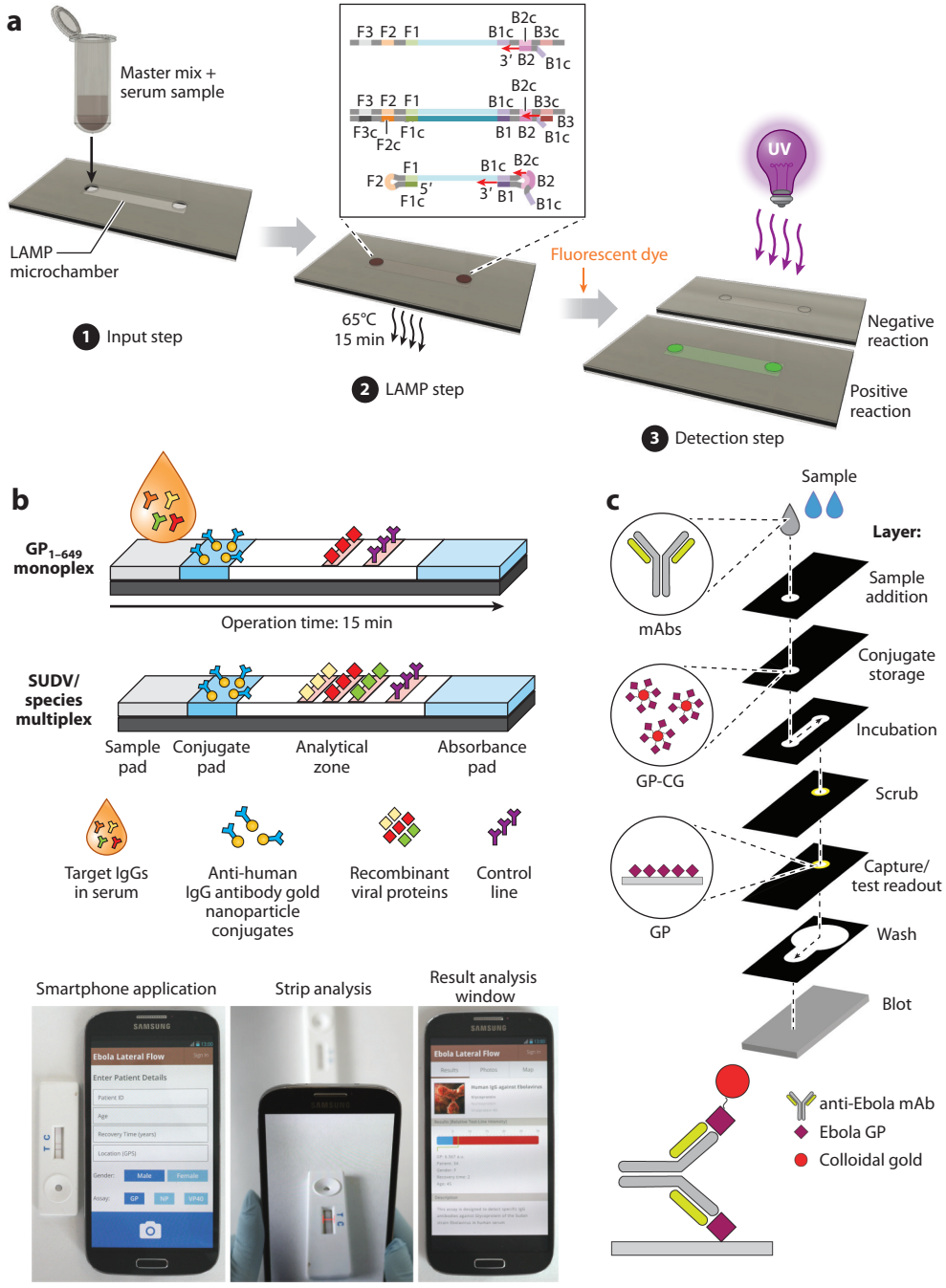

Figure 3

(*a*) Reverse transcription loop–mediated isothermal amplification in polyester-toner microdevices for dengue detection and (*b*,*c*) microfluidics devices for detection of Ebola virus. (*b*) A lateral flow strip for detection of IgG from the virus using a smartphone. (*c*) A paper-based immunoassay for Ebola virus surveillance. Abbreviations: GP, glycoprotein; GP-CG, glycoprotein-colloidal gold; IgG, immunoglobulin G; LAMP, loop-mediated isothermal amplification; mAb, monoclonal antibody; SUDV, Sudan virus. Panel *a* adapted from Reference 80 (CC BY 4.0); panel *b* from Reference 100 (CC BY 4.0); and panel *c* from Reference 104, copyright 2021 Springer Nature.

LODs of 200, 46.7, and 74.8 ng mL^{-1}, respectively. The authors reported that the use of a μPAD provided satisfactory specificity and sensitivity for detection of dengue NS1 in serum samples collected from pediatric patients infected and clinically diagnosed with the disease.

Alejo-Cancho et al. (83) evaluated a new rapid and semiquantitative microfluidic DENV NS1 immunomagnetic agglutination (IMA) assay based on the aggregation of magnetic nanoparticles detected by an electronic reader (such as ViroTrack Dengue Acute and BluBox from BluSense Diagnostics). The results were compared to conventional ELISA and an immunochromatographic test. The IMA assay exhibited higher sensitivity than the immunochromatographic test and slightly lower sensitivity than ELISA. However, the proposed method emerges as an alternative powerful assay for rapid dengue diagnostic testing based on NS1 detection because it provides results within 15 min through a semiquantitative measurement and uses a small volume of sample (30 μL). A comparative study was performed by Wongsawat and colleagues (84), who achieved similar performance of the IMA assay compared to ELISA and better performance compared to immunochromatographic test for early and rapid diagnosis of DENV infections in endemic countries.

Maeno et al. (85) reported the use of microfluidic immunoassay device already reported by other research groups (86–88) called 3D Stack for ELISA for the detection of the soluble antigen CD163 (sCD163), an indicator for DENV. The device consists of a microfluidic channel manufactured by stacking five polyethylene terephthalate (PET) films using a press, with a distance of 20 μm between the polymers, which produces the microchannels to increase the contact surface of the capture antibodies immobilized on the device. Subsequently, the device is rotated in an ELISA plate to promote the flow of liquid within the microchannels, thus ensuring higher amounts of antibody–antigen binding. The 3D Stack's characteristics were optimized and its performance was evaluated for sCD163 detection using sera from patients infected with DENV.

Low-cost microfluidic devices have been also developed to promote the detection of other viruses such as ZIKV and CHIKV. Kaarj et al. (89) developed wax-printed μPADs for ZIKV detection based on RT-LAMP. The color change was seen within 15 min, and quantitative information was extracted through smartphone imaging. The device was evaluated for different sample matrices, such as tap water, human urine, and human plasma. The achieved LOD was 1 copy μL^{-1}, and the device can be studied to detect other targets in addition to ZIKV.

Most recently, Wheeler's group (90) demonstrated the potential of DMF as a sample processing platform for ZIKV nucleic acid testing. The DMF platform involves five main steps: sample lysis and reversible RNA capture on magnetic beads, RNA extraction, RNA cleanup, isothermal amplification of Zika RNA, and detection. The platform was successfully used for testing lab-grown ZIKV in Recife, Brazil.

Theillet et al. (91) developed a laser-cut μPAD for detecting CHIKV IgM. In their study, chikungunya pseudo particles were used as capture antigens for detection of virus-specific IgM in human serum samples. As a proof-of-concept, the device provided a response within 10 min with a sensitivity of 70.6% and specificity of 98%. A disadvantage of this method is that the sensitivity of the proposed μPAD is lower than that of standard ELISAs. Although ELISAs have offered high sensitivity, commercial kits do not match the ASSURED criteria. On the other hand, μPADs are real candidates to replace gold standard techniques once they have the capability of providing faster analysis with reduced consumption of samples and reagents and at much lower cost per unit or assay.

2.2.2. Ebola. The Ebola virus (EBOV) has caused thousands of deaths (11,308 deaths as of this writing) and is highly infectious because of its transmission through body fluids. The main symptoms are fever, fatigue, headache, muscle aches, chills, diarrhea, and internal bleeding

(92–95). The EBOV genus has five species, named according to the area where the fatality occurred, such as Zaire (EBOZ), Sudan (SUDV), Côte d'Ivoire's Taï Forest (TAFV), Bundibugyo, Uganda (BDBV), and Reston, Virginia (RESTV). Among these, EBOZ is the main causative agent of Ebola disease (96). The disease is endemic in Central and West Africa, and its last outbreak was in 2014 in West Africa (97).

Hu et al. (98) developed a lateral flow assay for detecting EBOV glycoprotein using a novel multifunctional nanosphere. The device was manufactured by adhering gold nanoparticles (AuNPs) onto the surface of red fluorescent nanospheres (RNs). The authors reported an enhanced analytical performance in terms of colorimetric and fluorescence responses due to the presence of hundreds of quantum dots and dozens of AuNPs into each multifunctional nanosphere (RNs@Au). The proposed lateral flow device could be used by an analyst to detect with the naked eye EBOV in spiked urine, plasma, and tap water samples within 20 min. The authors successfully demonstrated the device's capability for POC assays in the field.

The development of ready-to-use μPADs for nucleic acid amplification–based tests for detecting EBOV was reported by Magro et al. (99). Isothermal reverse transcription and recombinase polymerase amplification (RT-RPA) of a synthetic ribonucleic acid of EBOV using paper microfluidics devices were performed, and the results were obtained within a few minutes with a sensitivity of 90.0% for a group of 43 EBOV-infected patient samples.

A smartphone lateral flow POC test for EBOV IgG detection was described by Brangel and colleagues (100). The prototype includes an immunochromatographic strip and a smartphone reader for Ebola-specific antibody detection. The detection methodology was based on the formation of complexes between the labeled AuNPs and the target analytes. Results were obtained within 15 min, and a positive result was indicated by a visual red-purple line formed at both the test and control lines (**Figure 3*b***).

Fernández-Carballo et al. (101) developed a microfluidic, real-time, fluorescence-based, continuous-flow quantitative reverse transcription-polymerase chain reaction (qRT-PCR) technique for EBOV detection. The disposable microfluidic chip was manufactured in a thermoplastic cycloolefin polymer, with the potential to be produced in large quantity through industrial prototyping protocols like roll-to-roll UV embossing manufacturing. The device tested by the authors with EBOV provided amplification faster than most conventional qRT-PCR platforms and commercial EBOV detection assays, with similar sensitivity (10 RNA copies μL^{-1}) and efficiency (90–110%).

A microfluidic chip with a portable RT-LAMP system was developed by Lin et al. (102) for the rapid and specific detection of four EBOV species: EBOZ, SUDV, BDBV, and TAFV. The system comprised four virus-specific LAMP primers, a microfluidic disk chip made of poly(carbonate) by the precision injection molding technique, and a portable real-time fluorescence detector. The chip showed specificity among viruses: LODs of 100 copies μL^{-1} for EBOV, 1,000 copies μL^{-1} for BDBV, and 10 copies μL^{-1} for SUDV and TAFV. Other remarkable features of the device include low sample and reagent consumption (0.94 μL) and a relatively short detection time (50 min).

Qin et al. (103) performed automated and rapid detection of EBOV RNA using RNA endonuclease Cas13a through clustered regularly interspaced short palindromic repeats (CRISPR). This system is based on fluorescent measurement of RNAs resulting from the nonspecific cleavage of quencher when it is bound to a target viral RNA. This automated system showed potential for virus detection because it was able to provide a response within 5 min, with low sample consumption and without using solid-phase extraction. This device can also be used for POC clinical diagnosis of other viruses by changing the complementary CRISPR RNA (crRNA) spacer sequence programming for different analytes.

Using the glycoprotein ectodomain of the EBOV envelope as an affinity reagent, Murray and coauthors (104) proposed a μPAD to diagnose EBOV based on a single assay to detect anti-Ebola IgG found in patients during the second week of acute illness (**Figure 3*c***). The authors observed that the affinity and binding rate of antibodies for the glycoprotein ectodomain influenced the analytical sensitivity. They found that the monoclonal antibodies having binding affinity around 3–25 nM revealed lower LODs, approximately 1 μg mL^{-1}. This shows the importance of the quality of the recognition agents used in POC tests for achieving desired analytical performance.

2.2.3. Rabies. The rabies virus has a small negative-stranded RNA genome encapsulated into the nucleoprotein (105). This virus uses neuromuscular circuits (NMCs) to invade the central nervous system at the neurovascular junction of the hypothalamus, causing flaccid paralysis and death (106). Some reported studies involve microfluidic platforms for modeling NMCs. These facilitate possible disease modeling and drug discovery, as rabies is considered a tropical disease and the most lethal among viruses (107, 108).

Bellmann et al. (106) developed a customizable PDMS microfluidic platform for modeling human NMCs (**Figure 4*a***). For this purpose, the authors showed for the first time the proof-of-concept by using monosynaptic rabies tracing to detect NMCs of rabies. As the NMCs consist of two compartments, central nervous system and peripheral skeletal muscle, the authors designed the master microfluidic platform to contain structures with different heights; they had exactly 100 lines with a height of 3 μm, width of 6 μm, and length of 900 μm, connecting two squared pillars with heights of 400 μm and sides of 3.5 mm. The NMCs were stimulated using blue light pulses, which enabled the cells to be video captured and quantified by changes in pixels as a result of muscle movements.

A microfluidic chip was presented as a microenvironment platform by Bauer et al. (109) to establish functional neuromuscular junctions using human-induced, pluripotent stem cell–derived motor neurons and human myotubes. Although the reported device was fabricated using photolithography, it was designed to contain four cells interconnected by microsized channels only permissible to axons. The authors demonstrated the functionality of this platform for rabies virus retrograde monosynaptic tracing by analyzing the data obtained from electrophysiological recordings of human-induced pluripotent stem cell–derived motor neuron networks on microelectrode arrays.

Sundaramoorthy and coworkers reported two studies associated with rabies virus that used commercial microfluidic devices from Xona Microfluidics. In the first report (107), they described a complex study that showed the role of axonal degeneration mediated by sterile alpha and Toll/interleukin-1 receptor (TIR) motif–containing 1 (SARM1) in the rabies pathogenesis. The authors used a microfluidic-based ex vivo neuronal model to show that SARM1-mediated axonal degeneration impedes the spread of the virus between interconnected neurons. Nonetheless, the neural defense mechanism resulted in the loss of both axons and dendrites. The viral titers of inocula were quantified by a direct fluorescent antibody test in BHK cells. The authors demonstrated the potential host-directed mechanism behind neurological dysfunction in rabies infection. In the second report (110), the authors developed a stem cell–delivered ex vivo model to study viral replication. The study introduced a system to evaluate pathogenic characteristics of rabies in human neurons, a method that can help identify cellular mechanisms associated to this disease and complement in vitro models.

2.3. Helminthic Diseases

According to the WHO, helminth infections are common, affecting approximately 24% of the world's population, principally the poorest and most socially vulnerable communities with low

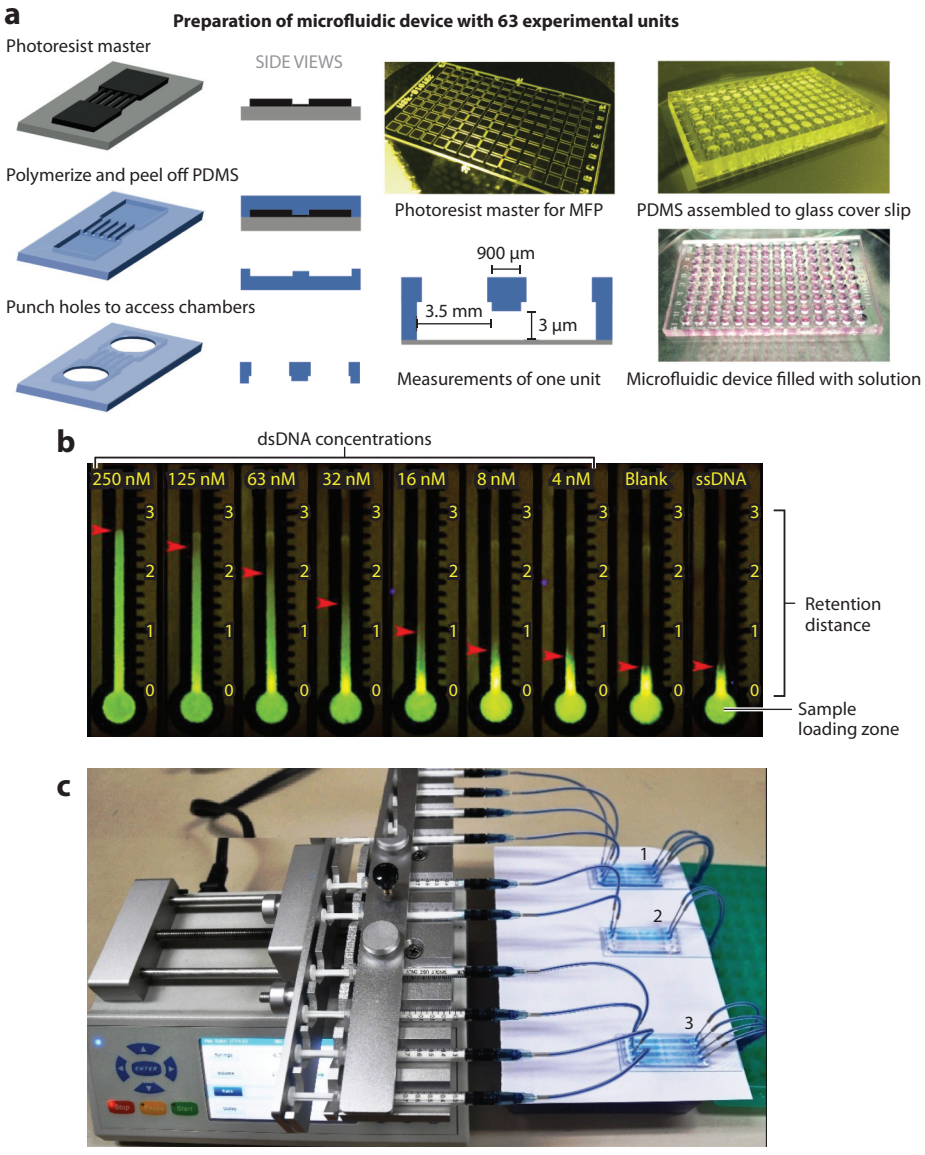

Figure 4

(*a*) Microfluidic platform for medium-throughput modeling of neuromuscular circuits for rabies viral tracing. Panel adapted with permission from Reference 106 (CC BY-NC-ND 4.0). (*b*) Distance paper-based device for quantification of soil-transmitted helminth infections. The measurements are based on the retention distance (0–3) determined by visual detection (*red arrowheads*). Panel adapted with permission from Reference 113; copyright 2018 American Chemical Society. (*c*) Semiautomatic microfluidic device using high-resolution melting real-time PCR to diagnose lymphatic filariasis. An infusion pump with syringes and blunt-ended needle are connected to 1, 2 and 3 inlet of the microfluidic chips. Panel adapted from Reference 116 (CC BY 4.0). Abbreviations: dsDNA, double-stranded DNA; MFP, microfluidic platform; PCR, polymerase chain reaction; PDMS, polydimethylsiloxane.

sanitation quality. Generally, helminths are transmitted by eggs present in human feces. The symptoms may vary according to the stage of infection, and light-intensity infections generally show no symptoms; however, heavy infections can cause intestinal manifestations, malnutrition, general malaise, weakness, and impaired growth and physical development. Very high-intensity infections can cause intestinal obstruction that should be treated surgically (**https://www.who.int/**).

2.3.1. Ascariasis. *Ascaris lumbricoides* is a soil-transmitted helminth infection that affects poor and deprived communities worldwide. The transmission generally occurs through contact with contaminated soil in areas lacking sanitation or by reusing water from wastewater depuration. The helminths' ova are capable of surviving for months in water and years in soil, so ascariasis is a concern principally for communities where wastewater and biosolids are reused (111). This tropical disease affects over 819 million people globally. Children under ten years old are the most prone to infection that can cause nutritional harm in multiple ways, for example, anemia, cognitive impairment, malnutrition, and gastrointestinal or pulmonary complaints (112).

Wang et al. (113) developed a distance paper-based device for quantifying soil-transmitted helminth infections. The device was fabricated with a wax-printing protocol, and the assay principle for nucleic acid analysis was based on interactions between SG-I and cellulose. The authors observed two important factors. First, the binding affinity affects the elution of SG-I into the test zone by double-stranded DNA (dsDNA). Additionally, the SG-I elution strength depends on the dsDNA concentration, facilitating the quantitative determination of dsDNA through the distance traveled by SG-I in the test channel. Second, the SG-I fluorescence was increased after the cellulose binding. Thus, it was possible to observe the distribution of SG-I inside the device in the presence or absence of dsDNA (**Figure 4*b***). In this study, the authors analyzed genome samples isolated from *Trichuris trichiura* and *A. lumbricoides* worms from children who had received chemotherapy.

2.3.2. Lymphatic filariasis. According to the WHO, lymphatic filariasis, usually known as elephantiasis, is a lymphatic system infection transmitted by mosquitoes that carry filarial parasites. Lymphatic filariasis is a helminthic infection that can be asymptomatic, acute, and chronic. However, most of these cases are asymptomatic, which contributes to the spread of the parasite through the lymphatic system, kidneys, and immune system. In general, the physical deformities caused by this illness cause social problems due to its effect on people's mental health, the loss of income-earning opportunities, and high medical expenses (114, 115).

In this context, Phuakrod et al. (116) proposed a semiautomatic microfluidic device to detect circulating microfilariae using high-resolution melting real-time PCR (**Figure 4*c***). The device was used to diagnose lymphatic filariasis in humans because it allows rapid and high-throughput detection and identification of this virus in blood samples. The authors produced a microfluidic device with photolithography using the polymeric material PDMS. The assay could analyze up to 10 blood samples simultaneously. A total of 384 samples were tested in this study. Thus, the chip proposed by the authors with PCR coupling presents a fast, reliable, and low-cost assay for diagnosing filarial parasites in samples from cats.

In 2021, two groups reported on microfluidic devices for low-cost diagnoses of lymphatic filariasis. Loymek et al. (117) proposed an investigation to check the prevalence of canine microfilaremia using a novel microfluidic device in combination with real-time PCR. In this work, the authors demonstrated that this device enabled easy and rapid high-resolution melting real-time PCR and provided useful insight into the risk of zoonotic filarial transmission from the cohabitation of infected animals and humans.

Phuakrod et al. (118) proposed a portable and low-cost miniPCR-Duplex lateral flow dipstick platform to visually and rapidly diagnose lymphatic filariasis infections. The devices used

here were the same as those reported by the authors in 2019, mentioned earlier. The analysis cost of each sample was estimated to be around US$5. The device showed promising results that facilitate rapid diagnosis at the collection site and for the first time could detect both *Brugia malayi* and *Wuchereria bancrofti*, parasites that provoke the lymphatic filariasis in human blood samples.

2.4. Bacterial Diseases

The most common bacterial NTD is leprosy, which is a chronic infectious NTD caused by *Mycobacterium leprae*. This bacteria affects the skin by invading Schwann cells and attracting macrophages, causing tissue wounds. It also affects the peripheral nerves, mucosa of the respiratory tract, and the eyes, making the bacteria pathogenic. This is called type 1 leprosy, characterized by abrupt episodes of intense, localized, delayed-type hypersensitivity to *M. leprae* in the skin and/or nerves, whereas type 2 is correlated with a systemic reaction involving a cytokine storm and deposition of immune complexes on the skin and in organs. Leprosy is commonly transmitted through droplets expelled from the nose and mouth of infected but untreated individuals (119, 120).

Rêgo et al. (121) used qPCR to screen genes related to the immune response in leprosy RNA-derived peripheral leukocytes of patients with and without leprosy reactions to profile expression signatures correlated to type 1 or type 2. For this purpose, medium-throughput qPCR using the microfluidic system Biomark was employed. All analyses were based on real-time fluorescence accumulation data. The results showed a signature for type 1 in the blood encompassing genes of innate immune responses. However, with regard to leprosy reactions of erythema nodosum leprosum (type 2), only the *PARK2* gene of Parkinson's disease was significantly more expressed in leukocytes from patients with type 2.

Levels of targeted antibodies and biomarkers that can be detected in fingerstick blood were investigated by Corstjens et al. (122). The test for humoral and cellular biomarkers was used to diagnoses *M. leprae* infection. The quantitative assays were accomplished within lateral flow strips employing particles of up-converting phosphor (UCP) to visualize results via color change. The authors also evaluated the applicability of the fingerstick test for C-reactive protein that is described in the literature as a biomarker for active tuberculosis and Crohn's disease and that shares genes with leprosy. The readouts were obtained by a portable reader and the correlation between fingerstick blood and serum was investigated with leprosy patients from Bangladesh, Brazil, The Netherlands, and South Africa. The authors demonstrated that the test is compatible with fingerstick blood, which allows rapid, near-patient testing and monitoring of treatment without highly trained workers.

Silva et al. (123) investigated age-related alterations associated with $CD8^+$ cells in elderly leprosy patients and whether the immune response could influence leprosy pathogenesis. They employed a microfluidic-based qPCR technology from Biomark. After evaluating young and elderly patients, the authors observed an accumulation of memory in $CD8^+$ cells only in skin lesions of elderly patients when compared to young people, and this reinforces the hypothesis that these cells have increased in subpopulation during aging. The authors also found changes in the gene expression of the lymphocyte-activation gene 3 (*LAG3*) and programmed cell death protein 1 (PDCD1) receptors in cutaneous lesions of young multibacillary leprosy patients but not in elderly leprosy patients. This may indicate that the receptors are involved in mechanisms that generate a weaker response to the T lymphocytes in patients with this type of leprosy. Thus, this study generally showed that alterations associated with the age of the subpopulations of T lymphocytes can facilitate leprosy progression, which can be extended to other chronic infectious diseases.

3. CONCLUDING REMARKS

This review has summarized the latest advances in the development of low-cost microfluidic devices for detecting NTDs. Because the COVID-19 pandemic is still affecting the globe, a review on NTDs is timely. The focus on tropical diseases raises awareness of other disease outbreaks responsible for thousands of deaths worldwide. In addition, the capability of low-cost microfluidic devices to provide a self-diagnostic in a matter of minutes, with low cost and reduced consumption of reagents and samples, may represent a true contribution toward increasing life expectancy in developing countries. Thus, we believe this review of essential technologies potentially has a societal impact.

Considering the current state-of-the-art techniques, we can expect future advances in smart microfluidic devices for screening multiple biomarkers and simultaneously attempting to obtain a precise and accurate diagnosis, especially for those diseases that have similar symptoms. Another important issue is the concept of a so-called "diagnostics for all." As discussed in this review, the use of globally affordable and zero-cost materials can popularize and accelerate the development of new diagnostic tools that can be used in low-resource settings. Advances in this area are useful for the promotion of early diagnostic tools for tropical diseases and use similar tools to those developed for diagnosing COVID-19.

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