HHS Public Access

Author manuscript

Biosens Bioelectron. Author manuscript; available in PMC 2019 October 15.

Published in final edited form as:

Biosens Bioelectron. 2018 October 15; 117: 112–128. doi:10.1016/j.bios.2018.05.050.

Point-of-care microfluidic devices for pathogen detection

Behzad Nasseri[a,b], **Neda Soleimani**[a], **Navid Rabiee**[c], **Alireza Kalbasi**[d], **Mahdi Karimi**[e,f,g,*], and **Michael R. Hamblin**[g,h,i,*]

[a]Departments of Microbiology and Microbial Biotechnology and Nanobiotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

[b]Chemical Engineering Deptartment and Bioengineeing Division, Hacettepe University, 06800 Beytepe, Ankara, Turkey

[c]Department of Chemistry, Shahid Beheshti University, Tehran, Iran

[d]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

[e]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran

[f]Department of Medical Nanotechnology, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

[g]Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

[h]Department of Dermatology, Harvard Medical School, Boston, MA 02115, USA

[i]Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, USA

Abstract

The rapid diagnosis of pathogens is crucial in the early stages of treatment of diseases where the choice of the correct drug can be critical. Although conventional cell culture-based techniques have been widely utilized in clinical applications, newly introduced optical-based, microfluidic chips are becoming attractive. The advantages of the novel methods compared to the conventional techniques comprise more rapid diagnosis, lower consumption of patient sample and valuable reagents, easy application, and high reproducibility in the detection of pathogens. The miniaturized channels used in microfluidic systems simulate interactions between cells and reagents in microchannel structures, and evaluate the interactions between biological moieties to enable diagnosis of microorganisms. The overarching goal of this review is to provide a summary of the development of microfluidic biochips and to comprehensively discuss different applications of microfluidic biochips in the detection of pathogens. New types of microfluidic systems and novel techniques for viral pathogen detection (e.g. HIV, HVB, ZIKV) are covered. Next generation techniques relying on high sensitivity, specificity, lower consumption of precious reagents, suggest that rapid generation of results can be achieved via optical based detection of bacterial cells. The introduction of smartphones to replace microscope based observation has substantially improved cell detection, and allows facile data processing and transfer for presentation purposes.

*Corresponding authors at: Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA.

Keywords

Microfluidics-based assays; Point-of-care devices; Smart-phone based sensors; Low resource environments; Micropattern soft lithography; Lab-on-a-chip diagnosis; Infectious microorganisms; Biomarkers of infection

1. Introduction

The early detection and monitoring of diseases necessary to optimize treatment, reduce mortality, and improve overall cost-effectiveness. Rapid and more accurate diagnostic technologies/tools are key issues in the identification of pathogenic microorganisms including prokaryotic bacteria, eukaryotic fungi, viruses, parasites, prions and protozoa for clinical diagnostic purposes (Na et al., 2018). Microfluidic systems are automated manipulation devices containing microchannels allowing liquid flow (10–100μm), and several other components including micro-pumps, micro scale inlet valves and miniaturized outlet drains combined with different apparatus for analysis of the contents (Park et al., 2011; Sin et al., 2013). Microfluidic chips have been deployed to track pathogens, identify food-based microorganisms, as well as to discover new antimicrobial agents (Lei, 2012). Microfluidic systems represent a new generation of conventional detection methods that rely on steps including specimen preparation, reagent manipulation, bioreaction and detection, which can be integrated in a unique platform. The benefits of microfluidic systems for diagnostic purposes are rapid detection, ease of use, cost effectiveness and high accuracy in the identification of infectious diseases (Huang et al., 2017), including the most dangerous pathogens like HIV, HBV, and ZIKV. The use of microfluidic chips in medicine significantly decreases the time gap between detection and institution of clinical treatment, this being highly important for patient survival (Chiu et al., 2017). The advantages offered by portable microfluidic biokits can be especially significant in regions with poor health services. The conventional pathogen diagnostic methods require the isolation and the purification of large number of pathogenic cells in addition to other requirements related to cell culture. According to the guidelines, the time required for the different steps of the pathogen identification, e.g. cell cultivation, enzymatic amplification and polymerase chain reaction (PCR) is at least 3 days (Song et al., 2017). While the time spent to execute all these steps might be considered reasonable in poor service regions, in large cities with more advanced health centers, implementation using point-of-care (POC) techniques is required. The application of a portable diagnostic apparatus on a miniaturized scale reduces the costs considerably; as well as the time required for diagnosis, the length of hospitalization and the mortality rate in patients with infectious diseases (Wang et al., 2017). The use of new generation biosensors to track and detect biological factors has been progressively implemented over the past two decades. The high performance, small size and portability of microfluidic systems complemented with the capability to measure and optically monitor the chips has enabled an optimized diagnostic approach that has enormous advantages when compared to conventional techniques. Microfluidic systems with their biodegradable materials can rapidly implement the tests without the need for expert operators.

The first miniaturized microfluidic device was developed by Terry and his research team in 1970 at Stanford University (Terry et al., 1979). The techniques for analyzing aqueous solutions were subsequently developed by Manz and Becker (1997), Kopp et al. (1998), Chiem and Harrison (1997), Li and Harrison (1997), Salimi-Moosavi et al. (1997) and Liu et al. (1999), Simpson et al. (1998), Woolley and Mathies (1995). The ability to employ miniaturized microfluidic devices has allowed to make advances in the field of chemistry (analytical), biology (biochemistry and biomedical) and medicine (biomedical devices). Microfluidic systems are used in diverse fields such as optical diagnosis of pathogens and diseases, as well as in surface chemistry investigations. Rapid diagnosis by robust and portable “Lab-on-a-Chip” (LOC) devices have become important for the diagnosis of different diseases, particularly in resource-poor areas (Reyes et al., 2002). LOC diagnostic systems can be considered an alternative to a centralized laboratory because they can provide healthcare staff and outpatients with vital information on health-related issues, even in remote settings and in a real-time mode. LOC techniques can be considered complementary to accepted diagnostic procedures, especially in the setting of infectious diseases (Dittrich et al., 2006). Because of the simple, rapid and highly specific detection, LOC systems based on cell, DNA and proteins can help physicians to diagnose a wide variety of diseases and therefore choose the most effective therapy. Microfluidic diagnostic systems are constantly improving to ameliorate health care systems. These techniques use various forms of optical detection, to identify and quantify specific biomolecules in biological and medicinal approaches. These methods simplify the disease detection process while providing high specificity results and enabling reduction of expensive reagents consumption (Whitesides, 2006; Janasek et al., 2006). The development of these “microfluidic chips” has been particularly relevant in the microbiology field as well as in the diagnosis of infectious diseases repetition (Weibel et al., 2007). Some of the techniques that have been deployed include “photolithography”, “stereolithography”, “chemical diffusion lithography”, “physio-chemical lithography” and “3D printing”.

The behavior and properties of different microorganisms such as viruses, bacteriophages, fungi and bacterial cells have been exploited to monitor infectious diseases; for studying drug resistant microorganisms; and for exploring new therapeutic and diagnostic techniques. These medical diagnostic chips can take the form of microarray devices, microchannel biodevices, and microfluidic systems. The devices may have substantial differences based on their intended application, and based on the physical and biological properties of the organisms, such as their type, nature, cytomorphology and size. Their growth form such as free-swimming planktonic cells or biofilm-encapsulated cells is also an important factor to consider. Conventional microbiology culture techniques require the use of tubes, petri dishes, multiwell plates and flasks. More sophisticated devices such as “bioreactors” and biofilm-forming equipment have nutrient inlets and drainage outlets for exhausted medium. In these devices, gases like CO_2 and O_2, necessary for the growth of microorganisms, can be supplied by micropumps designed in microfluidic chips.

2. Design of microchannels in microfluidic systems and lab-on-a-chip devices

Most of the procedures used to develop microfluidics take advantage of concepts originally validated in the semiconductor field (Vilkner et al., 2004). Lab on Chip (LOC) platforms have aspects in common with microarrays and biosensors. The basic substrate necessary is often glass, quartz or silicon. However, in microfluidic devices, polymeric materials such as polytetrafluoroethylene (PTFE), photosensitive silicon, poly(methylmethacrylate) (PMMA) (Ionescu-Zanetti et al., 2005; Becker and Gärtner, 2008; Mukhopadhyay, 2007; Duffy et al., 1998) and biopolymers such as calcium alginate (Ertl et al., 2004) and cross-linked gelatin (Ertl et al., 2004; Chin et al., 2007) can be used as a skeleton for the microfluidic device. Miniaturized detection chips to detect nanomolar or picomolar concentrations of analytes on a silicon substrate provide the specificity necessary for biological studies (Morens et al., 2004; Harrison et al., 1992). Effective development of diagnostic systems such as LOC and microfluidics requires ultrahigh sensitivity and specificity to enable real-time analysis needed for medical diagnosis (Beebe et al., 2000; Lagally et al., 2000). These detection platforms (LOC and microfluidics) are generally combined with other equipment for data acquisition, signal processing and monitoring. Readouts can employ various optical techniques, magneto-resistive sensors (GMR), electrochemical detection, acoustic methods, mass spectrometry methods (MS), or nuclear magnetic resonance (NMR) (Zourob et al., 2005; Mujika et al., 2009; Li and SU, 2006; GODbER et al., 2005; Tamarin et al., 2003; Mason et al., 2003; Gomez-Sjoberg et al., 2005; Lee et al., 2008b; Silley and Forsythe, 1996; Boehm et al., 2007; Rider et al., 2003; Xiang et al., 2006; Yadavalli and Pishko, 2004).

Optical absorption suffers from weak signal intensity because of the limited path length typical of microfluidic channels (Yi et al., 2006). Other optical detection methods employ photo-diodes integrated with microfluidic systems or LOC devices. For instance, photodiode measurement of ATP bioluminescence for the detection of air bone microbes has been reported. Living microbial cells in a liquid medium can be detected by photodiodes, phototransducers or other optically sensitive devices (Hochstetler and Matosky, 2005). Fluorescence-based detection represents a common option in microfluidics for microbiological diagnosis and is based on fluorescently-labeled molecular recognition systems (antibodies, enzymes etc.). A wide range of fluorescent dyes have been used in optical detection protocols (microscopy) such as eosin, hematoxylin, phycoerythrin (PE) cyanine dyes, fluorescein isothiocyanate (FITC) and Alexa dyes (Gaits and Hahn, 2003). A novel example of an optical technique that can be coupled with the microfluidic systems is Raman spectroscopy (Chen and Choo, 2008; Cheng et al., 2010; SalmanOgli et al., 2016). The Raman dyes known as “Raman Reporters” include rhodamine 6G, rhodamine 123, 3,3′-diethylthiatricarbocyanine iodide (DTTC) and can be used when the targeted moiety does not supply sufficient signal in absorbance or fluorescence modes. The Raman reporter binding to the target analyte provides Raman scattering when excited by a suitable laser (SalmanOgli et al., 2017a).

The most important aspect of microfluidic systems used for pathogen detection is the design of the microchannels, the manufacture of the apparatus, as well as the identification and quantification of the biomarkers, biomolecules etc. These goals can be accomplished with very low sample volumes, which have to be supplied by injection using a micro-pump. Various methods are used in the manufacturing of microfluidics systems. Most of these methods can be categorized as different forms of soft lithography.

The different stages of manufacture include the design of the master, the production of a photosensitive mask and the intermediate and final patterning steps. In soft lithography, a defined pattern is imprinted on a photosensitive mask to allow the final pattern to be elaborated in the next stage. In the core technique of “photolithography”, an appropriate polymeric material such as elastomeric silicone (poly-dimethylsiloxane, PDMS) is often selected for the creation of the basic substrate (Anderson et al., 2000). In the following stage, a photosensitive material is used for the preparation of a mask. A liquid polymer is usually applied to the surface of the body. Then, the fabricated mask is exposed to UV irradiation to form the desired pattern of channels on the surface of the photosensitive lamina. The UV exposed layer is used as the master mask for imprinting the final pattern on the PDMS block (Xia and Whitesides, 1998).

3. Core steps in soft lithography

The core steps in this technique, soft lithography, have been summarized as: A) microcontact printing; B) micromolding of capillaries; C) replica molding; and D) microtransfer molding. These steps are illustrated in Fig. 1.

3.1. Microcontact printing

Microcontact printing is a form of photolithography used for the transfer of a defined template onto the PDMS stamp (Fig. 2A). The self-assembled monolayers (SAMs) accumulate from ink building up on the substrate via conformational contact as in the case of nanotransfer printing. The desired pattern is created on the PDMS body. The PDMS body can then be coated using molecular recognition systems such as antibody molecules that are immobilized on the surface of the substrate via physical forces, or covalently attached by chemical linkers etc. This stage is repeated for a small number of times for the fixation of additional moieties (second and third attachments), such as enzymes.

3.2. Micromolding in capillaries

The process known as “micromolding in capillaries” (MIMIC) is used to pattern a substrate surface with micrometer-scale structures. An elastomeric stamp constructed from PDMS and bearing relief features on its surface is placed in close contact with a substrate to form a network of interconnected channels. A liquid polymer precursor is allowed to fill these channels by capillary action. After the polymer has cross-linked, the elastomeric stamp is removed (Weibel et al., 2007). This technique is mostly used in cell biology research (Kim et al., 1996).

3.3. Replica molding

Replica molding is used for the duplication of templates, as it replicates the size and shape of the master pattern. In contrast to photolithography, replica molding reproduces the three-dimensional structure of the microchannel features. A pre-defined topographical pattern created on the surface of PDMS acts as a mold for the mirror image in this technique. Replica molding is a more convenient technique compared to other procedures as it uses repetitive light exposure in photolithography (Fig. 2C) (Xia and Whitesides, 1998).

3.4. Microtransfer molding (μTM)

The microtransfer molding (μTM) technique employs a thin film of liquified prepolymer applied onto the surface of the PDMS cachet. (Fig. 2D). The excess amount of prepolymer is scraped off, and the remaining PDMS features filled with prepolymer are cured. The cachet is peeled away leaving the pattern on the surface of the substrate (Weibel et al., 2007). The μTM technique is suitable for microfluidic involving microelectromechanical systems used in LOC devices. The benefits of μTM include the rapid procedure, the ability to create isolated and interconnected channel patterns and the possibility of using complex materials such as polymers doped with dyes.

4. Microfluidic devices for the detection of microorganisms

Microfluidic systems that can allow fluid flow in desired patterned channels are used to study the effects of drugs interacting with cells or other chemical-biological interactions. These devices are also used for the detection and quantification of pathogens without the need for cell culture. Microfluidic chips for this application can be classified into those interacting with bacterial cells (Tani et al., 2004), biomolecules (Zhou et al., 2012; Liu and Lee, 2005; Malic et al., 2009), pathogens (viruses) (Foudeh et al., 2012; Guan et al., 2010) and biomarkers (Lee et al., 2008a; Gervais et al., 2011). Various protocols and microfluidic systems have been designed and manufactured for the detection of biological moieties at the 2D and 3D levels. In these protocols, the ability to employ a small sample volume with appropriate levels of sensitivity and specificity is important. In conventional techniques such as immunoassays, which use multi-well plates for pathogen identification (ELISA), and PCR based pathogen identification systems, sample volumes, assay duration, and cost effectiveness are not necessarily prioritized. In conventional microbiology methods, the collection of the microorganisms, their culture and expansion are carried out separately from the analysis and the identification, while in microfluidic systems the chip can combine all these individual functions into a single complex system.

5. Optical biosensors for the detection of pathogenic microorganism

Diagnostic techniques in microbiology are generally divided into two main categories, either cell-culture based diagnostic protocols or molecular techniques that do not (necessarily) require cell culture (Tani et al., 2004). Cell culture protocols are used to detect pathogenic moieties and provide conventional information such as the type of medium needed to isolate and grow the cells. These become particularly useful in those situations when the diagnostic yield depends on the number of living bacterial cells, Dr. Robert Koch developed this

approach in the last quarter of the 19th Century (Zhou et al., 2012). The commonly used molecular methods include mass spectroscopy (MS) (Diamandis, 2004), chromatography, gel electrophoresis (Navarro-Noya et al., 2012) and enzyme linked immunoassay (ELISA) (De La Rica and Stevens, 2012). Despite the wide application range of cell-culture based diagnostics, there are drawbacks including the need for an expertly trained operator and the long time required (2–4 days).

Novel approaches such as optical, electrochemical, acoustic techniques and nuclear magnetic resonance (MNR), are included in the 'non-cell culture' diagnostic category. The most accurate and rapid methods amongst the non-cell culture procedures rely on optical techniques.

Due to their safety, rapidity and multipurpose capability combined with high sensitivity, optical biosensors are very promising in detection methods for pathogens (Yoo and Lee, 2016). The optical biosensors deployed in the diagnosis of microorganism can be categorized into substrate and sensor-based mechanisms. In the substrate-based procedures, paper (Pöhlmann et al., 2014), polymer (Carey et al., 2011), glass slides (Wang et al., 2012) and silicon wafers have been used as platforms. The substrates can be coated using optosensitive conductive layers such as magnetic beads and nanometer thicknesses gold layers as an optosensitive laminate to capture the pathogenic moieties such as *E. coli* cells (Tram et al., 2014). Other substrate groups can be Au plates (Tawil et al., 2012) and semiconductor materials e.g. Au-silica (core-shell) nanoparticles (NPs) applied for the detection of *P. aeruginosa,* and *S. typhimurium* (Yoo et al., 2015). The other types of biosensors include: copper coated silica core nanoparticles used in localized surface plasmon resonance (LSPR) mechanisms (Kim et al., 2011); dielectric materials; and carbon nanotubes (Yang et al., 2013). The most common substrate category used in the detection of microbes is represented by glass slides that are normally utilized for diagnosis of *P. aeruginosa* and *S. typhimurium* (Yoo et al., 2010). The different capturing moieties can be immobilized on the surface of substrates to diagnose *E. coli O157:H7.* In this group, antibodies (Wang et al., 2012), aptazymes (Tram et al., 2014) and DNA fragments participate in the tracking of *E. faecium, S. aureus, Vibrio vulnificus,* and *Stenotrophomonas maltophilia* (Kang et al., 2010), The aptamer enables specific capture of molecules particular to *E. coli, L. monocytogenes, S. enterica* and this can be exploited to target pathogens (Bruno, 2014) as well as *S. aureus, P. aeruginosa (*Wan et al., 2014). The wide application of substrate based diagnosis (e.g. lateral flow assay, LFA method) for pathogen detection relates to the easy applicability LFA, accessibility and limited costs. The use of this technique however, is limited due to its low sensitivity, consumption of large amounts of reagents and the time required. The template applied to the substrate relates to the desired detection methodology. For patterning of the substrate, various photolithography techniques such as inkjet printing, plasma etching (Malic et al., 2009; Foudeh et al., 2012; Guan et al., 2010) and wax printing can be used and numerous products are readily available as diagnostic kits. The LFA based biosensors have been manufactured and are currently provided by DuPont Co. The product marketed by DuPont Co. can detect *E. coli O157* and *Salmonella* within 10 min. Further, the BioMerieux diagnostic technology enables the detection of *Streptococcus* and *Legionella pneumophila* within 15 min using the LFA method. The substrate-based technique was discussed earlier and is also discussed in Section

5, and schematic illustration of this protocol are shown in Fig. 2A. The benefits of substrate-based techniques (LFA in cassette or card type chips) are a simple color change induced by the nanostructures (aggregation of the optosensitive nanoparticles such as AuNPs or AgNPs). In many studies the interaction between an antigen (pathogen as target), an antibody (receptor moiety) and nanoparticles (optical indicator) forming an NP-Antibody-Antigen bio-conjugate. The structure of the antigen-antibody-nanoparticle combination depends on the intensity of bioaffinity. The combination of pathogen-AuNPs-antibody generates a red color change (AuNP aggregates) in the presence of the pathogen. Instead of AuNPs, other nanoparticles such as magnetic beads can be used and these present several advantages. The magnetic beads are used to concentrate the pathogenic cells (*E. coli* bacterial cells) conjugated to the nanoparticles. The magnetic beads enable the rapid concentration of cells (10–100 fold) and are therefore used for diagnostic purposes (Huq and Colwell, 1996). The other category within optical based biosensing is represented by the plasmonic and signal amplification. In this group NPs such as AuNPs or quantum dots (QDs) (Gervais et al., 2011) are applied in optical sensor colorimetric techniques to detect pathogens (Ko et al., 2014; Jokerst et al., 2012; Funes-Huacca et al., 2012), or by taking advantage of color changes produced by gold nanoparticles (AuNPs) after aggregation (Bruno, 2014; Hossain et al., 2012). Magnetosome amplification, target recycling reactions, and DNAzyme-based reactions are examples of signal amplification applications. Other examples are provided by plasmonic-resonance-based AuNPs (Funes-Huacca et al., 2012). In addition to the AuNPs used in the LSPR-based diagnosis of biological moieties, other nanostructures like AuNRs and QDs (Diamandis, 2004) are used in microfluidic systems.

The diagnosis of pathogens by biosensors depends on the optical properties of the materials. Optical properties including plasmonic, local surface plasmon resonance (SPR), localized SPR (LSPR) and surface enhanced Raman scattering (SERS) are used. In SPR, an external source of energy (e.g. laser beam) makes resonant oscillations in the electrons of the conduction band of the material. Both SPR and LSPR are analytical techniques for the measurement of analytes and receptors in cell studies (Abadian et al., 2014). SERS is a surface-sensitive mechanism based on Raman scattering (an optical characterization of certain materials) by molecules absorbed onto the surface of metals or nanoplatforms such as gold or silver nanoparticles. The specific optical fingerprint of materials utilized in the SERS method has wide applications in the fields of optoelectronics, optical physics and medical diagnostics. Application of SERS has been also extended to biological analytical methods (Hoppmann et al., 2014; Dina et al., 2017), diagnosis of biofluids (Bonifacio et al., 2015) *a*nd in cellular analysis. Because of the high accuracy, rapid detection, potency and single-cell diagnostic capability, SERS can be applied to detect different microorganisms such as *E. coli, E. lactis, L. casei* and *M. morganii* (Fig. 2B). In the Raman scattering (SERS technique) the key components are the substrate and the Raman dye (Raman Reporter), which is conjugated to cells to enhance the scattered light. The light scattered from the surface of the targeted cell (bacterial cell or viruses) provides successful and accurate diagnosis in less than 5 min. The fluorescein isologs, e.g. Rhodamine (Rh) and derivatives such as Rh6G, Rh123, RhB as well as dyes like tetramethylrhodamine isothiocyanate (TRITC) and tetramethylrhodamine (TMR) are commonly used in optical based biological studies such as SERS. The emission wavelength (λ_{em} =517 nm) is located in the stimulation

range of AuNPs. In the Raman based diagnostic method, the incident laser beam excites the probes of interest combined with biological cells and Raman reporter conjugated AuNPs or AgNPs. This results in Raman shift enhancement due to scattering from the surface of single cells which can be detected by sensitive detectors. The Raman shift generated by the difference between AuNPs in the absence or presence of conjugated pathogens leads to the identification of the pathogens. The accuracy of the results is largely dependent on the concentrations of AuNPs or Ag-NPs, on the Raman dyes and on the cell type. Other parameters such as laser power and wavelength are key factors in determining the performance of SERS. SERS scattering (by Raman reporters) using gold, silver and QDs for signal enhancement can be achieved at very low concentration of cells (10^{-9} M). Hence, a diagnostic method based on label-free SERS can provide accurate optical detection in a short period of time without specimen preparation, as depicted in Fig. 2. In the above-mentioned techniques, different substrates can be employed. These include paper (filter papers and nitrocellulose membranes) or glass and silicon wafers, which are coated with noble metal nanolayers and nanostructures (nanoparticles, nanorods, nanotubes and nanowires) to serve as nanosensors for conjugation to microorganisms.

Fig. 2C illustrates an acid based microfluidic chip for the detection of pathogens. This is based on the application of fluorescent dyes and metal nanoparticles via chemiluminescence. Although this review is mainly focused on microfluidic systems, a brief introduction to the techniques deployed in the diagnosis of pathogens, is necessary. These approaches include colorimetric and plasmonic (optical) techniques used in microfluidic chips and in Lab-on-a-chip microarrays. Fig. 2C, depicts a microarray where thousands of DNA spots (oligonucleotides) are immobilized to identify the nucleic acid sequence of interest via an optical mechanism (chemluminescence substrate) (Joung et al., 2014). Using this approach different moieties such as nucleic acid (DNA, RNA), functional nucleic acid (aptamers, DNAzymes), monoclonal antibodies and even bacteriophages can be used. The detection of *Bacillus*, *influenza* and *Yersinia enterocolitica* has been based on this technique (Liu et al., 2006a, 2006b; Wang et al., 2008; Myers et al., 2006). As illustrated in Fig. 2C, a fluorescent dye can be applied as bioassay-detection reagent for pathogens, while the nanoparticles were conjugated with DNA or mRNA strands. Fluorescent assays have different formats based on whether fluorescent nanoparticles, fluorescent nucleic acids or fluorescent caspases etc., are used. The beam induced on the microfluidic chip/microarray platform encounters gold nanoparticles. The surface plasmon resonance property of the dye-conjugated gold nanoparticle enhances the excitation at defined wavelengths. The wavelength difference (Stokes shift) due to the propagation of the beam from the surface, can be registered by sensitive detectors. In different types of fluorescent-based diagnostic methods, the changes in fluorescent dyes can be detected by fluorescent spectroscopy. Fig. 2D illustrates diagnostic methods based on substrate and nanoparticles (generally silver or gold) conjugated with appropriate linkers that create a sensitive platform excited by a light source. The difference between incident and reflected light (light intensity measurement) enables the signal detection (Fig. 2D). The technique depicted in Fig. 2D has advantages such as high measurement accuracy in and a short time to carry out.

Enzyme-linked immunosorbent assay (ELISA) is a conventional cell-culture-based method used for the detection of microbes. Different from new generation technologies such as

microarrays and microfluidic systems, in ELISA a wide range of antibodies each with specific antigen bioaffinity for pathogens are used. Microorganisms like *E. coli, Listeria* spp*., Listeria monocytogenes*, *Salmonella, Shigella* and *Staphylococcal* enterotoxins can be detected. HIV causes one of the most dangerous chronic infections, and is detected by ELISA. The HIV ELISA detects the presence of host antibodies, which have been produced as a result of the infection. The micrometer scale of microfluidic systems is ideally suited for ELISA based assays. Antibodies against other important viruses like hepatitis B and Zika viruses can be also detected by ELISA. Another technique within the non-culture category is microfluidic PCR-based pathogen identification. The advantage of this identification method is that it does not require an intermediate cell culture stage.

For detection methods that do require an intermediate cell culture stage, microfluidics devices can be equipped with micro-channels that can supply nutrients (inputs) and remove bio-wastes. Additional channels may be needed for the system manipulation and for the implementation of the desired interventions. The biological species (e.g. pathogens, cells, toxins etc.) produce a signal (optical, thermal, mechanical, electrical etc.), that can be modified by the effects of drugs, tracing of toxins etc. Table 1 summarizes microfluidic systems studies conducted for the diagnosis of microorganisms.

6. Microfluidic systems as point-of-care diagnostic devices

New-generation hospital centers are equipped with centralized diagnostic laboratories containing high-tech analytical devices and clinical instruments, which are not feasible or affordable for many less-developed countries. Microfluidic systems can be considered as portable point-of-care devices, the so called “Doctors office in the home” and are deployed for the diagnosis of diseases and detection of pathogens. Epidemic infectious diseases in less-developed countries as well as endemic infectious diseases caused by bacteria, viruses or fungal pathogens are all of great relevance for the public health authorities (Yager et al., 2006). Microfluidic-based detection can employ nucleic-acid amplification, blood chemistry assays, flow cytometry and immunoassays. There are differences between these detection techniques including the time-required for the test, the amount of reagents necessary for performing the assays, the cost effectiveness, the reproducibility etc. Immunoassays are rapid and simple diagnostic platforms that can use a simple strip, in contrast to blood chemistry measurements that are complex and time consuming because they require measurements of 12–20 individual physiological parameters. The benefits of nucleic acid amplification relies on the need for a limited copy number of nucleic-acids to make diagnosis (Yager et al., 2006).

Every physician is familiar with the initial question asked during the first meeting with a patient: “Is my (blood) sample positive or not? followed by: “What is the severity of the disease or the prognosis?” In conventional microbiological techniques based on cell culture methods, expensive labor and reagents might be involved; this at times resulting in long time elapses before results are generated. In microfluidic chips (microassays) the results can be obtained and evaluated in few minutes. The benefits of recently developed microfluidic systems for the detection of diseases include rapidity, reliability, minimal costs and user friendliness.

In order to evaluate the relative efficacy of diagnostic protocols we need to have an accurate comparison between traditional and newly developed techniques. Novel microfluidics-based systems can rapidly detecting targets at very low concentrations, and in small sample volumes (μL, nL, pL, fL). They have applications in molecular biology, infectious disease management, food safety and biodefense (Jayamohan et al., 2017). The devices incorporate channels and chambers with dimensions of tens to hundreds micrometers (Foudeh et al., 2012). In these devices the microenvironment needs to be tightly controlled, particularly the temperature (Streets and Huang, 2013) and the mechanical stress (Jayamohan et al., 2017). For signal amplification, advanced techniques including optical reporters are often employed, e.g. fluorophore dyes, metallic nanoparticles, quantum dots, carbon dots and graphene dots.

7. Detection of bacterial cells by microfluidic systems

Bacterial detection is one of the most important applications in medicine, since in many cases the choice of antibiotics is dependent on the identification of the causal agent. A variety of different bacteria have been detected by microfluiidics-based devices; here we will discuss the detection of pathogens most commonly involved in human pathology; these include: *E. coli, Mycobacterium tuberculosis (TB), Salmonella spp* and *Vibrio cholerae*.

7.1. Detection of Escherichia coli

More than 80 million cases of food-borne illness are reported each year in the USA and accounting for about 9000 deaths (Stokes et al., 2001). *E. coli 0157:H7* is one of the pathogens most commonly involved in these deadly cases as it can easily be introduced into the food chain due to fecal contamination at various stages of the production process. The initial symptoms include diarrhea, abdominal cramps and fever; whereas in later stages hemolytic uremic syndrome (HUS) can occur and lead to death. Conventional antibiotics are mostly ineffective.

Detection of *E. coli* has been reported using both antibody-based and DNA-based techniques. Colorimetric-biosensing using *E. coli*-specific antibodies conjugated to gold nanoparticles (Ab-AuNPs) was used in a lateral flow assay (LFA) with a 10-min detection time. Capillary flow allowed the bacteria to cause agglomeration of the Ab-AuNPs producing a blue to red color shift on the paper strip. The low sensitivity of the LFA based system, however, was pointed out as a disadvantage. The assay sensitivity could be improved by magnetic bead amplification and pre-concentration of the bacterial cells (Yoo and Lee, 2016).

In Stokes et al. (2001) described a multilayer detection platform, in which *E. coli* cells were immobilized on a substrate and allowed to bind to Cy5 labeled antibodies; binding was detected by an array of photodiodes along with amplifiers, discriminators and logic circuitry. Gau et al. (2001) reported a micro-electromechanical (MEMS) biosensor characterized by a “Self-Assembled Monolayer” (SAM) surface. Multiple electrodes were deposited on a Si wafer and a monolayer of streptavidin was immobilized onto the Au electrode surface. Biotinylated single-stranded DNA (ssDNA) probes were then attached to the Au using thiols to capture rRNA from *E. coli*. A second set of fluorescein-conjugated ssDNA probes were

then added followed by an anti-fluorescein antibody conjugated to peroxidase. Enzymatic amplification allowed the system to detect 1000 *E. coli* cells without any need for polymerase chain reaction.

7.2. Detection of Salmonella spp

Salmonella spp. is generally known as a food-borne pathogen. Rapid, sensitive and cost effective detection methods are required for its diagnosis (Kim et al., 2015). Polymerase chain reaction (PCR) based methods represent old methods deployed for the detection of Salmonella; these approaches, however, require expert operators, therefore being highly expensive (Hossain et al., 2012; Hart et al., 2011). Novel biosensing techniques within the microfluidic systems category (Kramer and Lim, 2004) and impedimetric methods (Radke and Alocilja, 2004; Varshney and Li, 2007) have been employed for the detection of Salmonella. Kim et al. (2015) described a microfluidic chip based on an antibody-sandwich approach. An anti-Salmonella polyclonal antibody was covalently immobilized onto quantum dots and a second polyclonal antibody was attached to superparamagnetic particles. Quantum dots and cell-bound magnetic particles were introduced into Sections 1 and 2 of the microfluidic chip; a permanent magnet and a portable fluorometer were used to measure the fluorescence signal from the quantum dot nanoparticles attached to Salmonella in the samples.

A smartphone-based device has been used in combination with paper based microfluidic chips as a portable, easy to use, highly sensitive and accurate method for the detection of *S. typhimurium* (San Park et al., 2013). Each paper microfluidic channel was pre-loaded with submicrometer (920 nm) polystyrene latex particles conjugated to anti-Salmonella antibodies. Dipping the paper microfluidic device into the Salmonella suspension caused the antibody-conjugated particles to immunoagglutinate. The extent of immunoagglutination was quantified by evaluating Mie scattering from the digital images taken at an optimized angle and distance using a smartphone. An application was designed to allow the user to position the smartphone; then an image-processing algorithm calculated and displayed the bacterial concentration on the smartphone. The detection limit was at the single-cell-level and the total assay time was less than one minute.

7.3. Detection of Vibrio cholerae

Vibrio cholerae was discovered in 1854 and found to be the etiological agent of epidemic cholera, an acute intestinal infection. This bacterial cell with its biofilm forming ability is known to be resistant to chlorine and other disinfectants. Various approaches have been attempted to detect *V. cholerae* (Yildiz and Schoolnik, 1999; Kong et al., 2002; Koch et al., 1993; Fykse et al., 2007). Jarvius et al. (2006) described a microfluidic device relying on rolling circle amplification (RCA) and single molecule detection. RCA results in a single amplicon consisting of a long single-stranded concatemer DNA molecule that contains the sequence complementary to *V. cholerae* 16S rRNA. This sequence is repeated approximately 500–1000 times to produce a visible particle (diameter 1 μm). The microfluidic platform produced by high-throughput injection molding allowed for sensitive confocal fluorescence detection with high-magnification optics (Jarvius et al., 2006; Fire and Xu, 1995). Fig. 3 shows a schematic illustration of this methodology.

In another study, Fykse et al. (2007) reported the detection of *V. cholerae* using real-time Nucleic Acid Sequence-Based Amplification (NASBA). NASBA has been successful for the detection of microbial cells in food. In the present work, a real-time multi-target NASBA assay was developed to apply for high specific detection in the case of V. cholerae cells. Molecular beacons signals and Primers were targeted to five different genomic sequences.

In another study, Batalla et al. (2015) used an electrochemical microfluidic chip for the separation and tracing of D-amino acid (D-AAs) such as D-Methionine (D-Met) and D-Leucine (D-Leu), the molecules which act as biomarkers related to diseases caused by *Vibrio Cholerae*. One of advantages of this study relied on the application of a simple chip of an electrochemical microfluidic system with low biological objects consuming. The microfluidic chip had the capability to separate DAAs beside the desired reaction between each D-AA biomarker and the D-amino acid oxidase (DAAO). One of the benefits of the applied microfluidic chip is the high yield enantiomer separation without consuming additives; this prevents covalent immobilization of enzymes on the surface of the chip's channels. Using this technique, successful separation of DAAs was achieved and in situ screening of *V. cholera* was carried out.

7.4. Detection of Mycobacterium tuberculosis (TB)

Tuberculosis is the ninth most common cause of death worldwide accounting for 10.4 million new cases in 2016 and 1.7 million TB-related deaths. Further, TB is the one of the most frequent cause of death in HIV infected individuals. The limited effectiveness of antibiotic treatment and difficulty in obtaining an accurate diagnosis has led researchers to search for better detection modalities. Different techniques such as gene-probe RNA testing, interferon-gamma assays and urine-based antigen test have been proposed as new TB tests (McNerney and Daley, 2011; O'Sullivan et al., 2002).

Scientists at Massachusetts General Hospital (MGH) have developed a method to provide rapid detection of TB and other bacteria (Liong et al., 2013). The detection technique was originally devised for identifying cancer cells (Ghazani et al., 2013; Haun et al., 2011); subsequently it was found to be applicable to bacterial detection and identification (Lee et al., 2008a). The portable rapid microfluidic device was based on nuclear magnetic resonance (NMR) detection, which was termed "diagnostic magnetic resonance. DMR" (Shao et al., 2012). DMR was based on superparamagnetic iron oxide nanoparticles. In assemblies with small magnetic nanoparticles (MNPs) (< 20 nm) the thermal fluctuations can overcome the anisotropy barrier and spontaneously flip the magnetic moment of each particle (Bean and Livingston, 1959). AS a consequence of this, an ensemble of MNPs possesses no permanent magnetism in the absence of external magnetic fields; the magnetic moment, however, grows with increasing external magnetic field to provide superparamagnetism. This ensures that MNPs do not spontaneously aggregate under physiological conditions. An MNP is typically comprised of an inorganic magnetic core with a biocompatible surface coating, which can be modified with functional ligands. MNPs efficiently destroy the coherence of the spin-spin relaxation of water protons. The derived net effect is a change in magnetic resonance signal, shortening of the longitudinal $T1$ and transverse $T2$ spin-spin relaxation times (Shao et al., 2012).

DMR detection exploits targeted MNPs to modulate the spin-spin *T2* relaxation time of biological samples. DMR assays are present in two forms depending on the size of the target. For the detection of small analytes, a method called magnetic relaxation switching (MRSw) can be exploited. When MNPs cluster in solution, the aggregates will assume different *r*2 values, causing corresponding *T*2 changes. MRSw assays are performed without removing the excess of unbound MNPs, therefore allowing the detection of small molecules. For larger biological targets such as bacteria, the cells can be tagged with functionalized MNPs; then, unbound MNPs are removed. Miniaturized NMR coils (so called μNMR (Lee et al., 2008a)) can be incorporated into microfluidics chips (Chung et al., 2013). Effective temperature control was a major challenge when moving DMR into a POC device due the inherent temperature sensitivity of the magnetic field generated by the permanent magnet. In a new device called μNMR-3, the Authors employed an electronic approach using a feedback routine that automatically tracks the temperature changes and correspondingly reconfigures the measurement settings (Issadore et al., 2011).

The system was configured to detect MTB (Liong et al., 2013). Polymeric beads were conjugated with MNPs attached to oligonucleotide sequences specific for MTB. The probes used synthetic 92-nucleotide (nt) single-stranded DNA (ssDNA) specifically found within the acyl-CoA de-hydrogenase fadE15 gene of MTB (Manganelli et al., 1999; Muñoz-Elías et al., 2005). Sputum samples were mechanically liquefied and amplified using on-chip PCR, then the MNPs and buffer solutions were loaded into separate chambers gated by valves. The PCR products were combined with MNP-labeled capture beads specific for the target amplicons. Changes in the transverse relaxation rate (DR2) were measured using μNMR. The sample volume per measurement was 1 μL. During experimental studies, ss-DNA and ds-DNA samples were compared. Ss-DNA was found to be superior as ds-DNA needed additional denaturation and annealing steps.

In another study so-called “cannonballs, CBs” coated with monoclonal antibodies were deployed (Lee et al., 2009). The CBs had a thin ferrite shell with very high r2 values produced by thermally decomposing $Fe(CO)_5$, then oxidizing the particles in air to grow the ferrite relatively thin shell (compared to chemical oxidation (Peng et al., 2006)). The antibodies recognized *Bacillus Calmette-Guerin* (BCG), used as a surrogate for MTB. A chip-based NMR compatible filter system consisting of a microfluidic chamber enclosed by a membrane filter and surrounded by a microcoil was developed. This not only captured and concentrated bacteria but also allowed on-chip separation of bacteria from unbound CBs. In this study, the Authors were able to detect as few as 20 colony-forming units in 1 mL sputum in less than 30 min.

A group from the National Microelectronics Research Centre (NMRC) in Ireland investigated the detection of MTB using thermo-cycling silicon micro-reactors in a microfluidic chip to amplify the DNA (Ke et al., 2004). PCR conditions were determined using Lambda bacteriophage DNA as a model reaction system. These devices were used to amplify a specific fragment of the rpoB gene from MTB, where mutations leading to multi-drug resistant strains are most likely to occur. Amplification was achieved in 34 min, compared to the 62 min required when using a conventional Peltier-heated PCR machine.

8. Detection of viruses using microfluidic systems

Viruses are micrometer scale pathogens that cause a range of diseases to humans, animals, plants and even microorganisms. These protein-coated nucleic acid-based pathogens are only able to replicate inside living cells. Viruses can be single or double stranded and composed of DNA or RNA and be highly infectious in some cases. Here we discuss three viruses as causal agents of serious diseases: human immunodeficiency virus (HIV), hepatitis B virus (HBV) and Zika virus (ZIKV).

8.1. Detection of HIV using microfluidic chips

According to the WHO, approximately 33.2 million people are infected with human immunodeficiency virus type 1 (HIV-1) worldwide. In 2016, 1 million people died from HIV/AIDS related diseases (HIV/AIDS, 2017). HIV is a single-stranded, positive-sense, enveloped RNA lentivirus that belong to the *Retroviridae* family. Upon entry into the target cell, the viral RNA genome is converted into double-stranded DNA by the enzyme reverse transcriptase, which is part of the virus particle. Common techniques for detection and monitoring of viruses in host immune cells are based on flow cytometry and quantitative PCR. These methods have the disadvantages of requiring a long time before results are known and involve expensive reagents, equipment and trained personnel (Fiscus et al., 2006). On the other hand, Point-of-Care (POC) devices need to detect approximately 200 $CD4^+$ cells μL^{-1} and 400 copies mL^{-1} of HIV in whole blood samples.

In another study, Wang et al. (2012b) used an integrated microfluidic system for HIV detection. They designed nucleotide probes to specifically bind the HIV particles and then attached them to magnetic beads. Subsequently, an on-chip PCR module was used to perform amplification of four detection genes (RU5, int, tat, and vpr) with the aim of increasing sensitivity and specificity and to avoid false negatives caused by the virus mutations. Detection of multiple HIV-1 fragments could be amplified successfully and detected in real-time using on-chip fluorescence within 95 min.

Chin et al. (2011) studied the detection and monitoring of HIV in Rwanda. They applied “mChip” assay on a tiny volume of patient blood (1 μL). The mChip showed great performance in the diagnosis of HIV. Unlike most current rapid diagnostic techniques, the mChip test did not require any specific user interpretation of the signal. The results provided by the miniaturized apparatus HIV (Kaur et al., 2000; Sackmann et al., 2014) proved the superior performance of the device and technique. Laksanasopin et al. (2015) introduced a new tool for virus detection. This consists of a “smart-phone dongle” to be deployed in less-developed countries like Rwanda. The microfluidic device was equipped with a smart-phone, a simple manual push button, a pump with 1 μL blood chamber and gold-labeled antibodies capable of targeting the pathogen of interest through a reagent cassette. The signal amplification was implemented by a silver layer, which the device was made of (Fig. 4). A particular panel was equipped with three different antibodies for a triplex immunoassay (anti-HIV, anti-syphilis treponeme and anti-cardiolipin non-treponeme). Whole blood obtained from 96 patients attending a mother-to-child transmission clinic allowed health care staffs to test and obtain results in 15 min. The results obtained using this microfluidic system showed that the device had the same specificity and sensitivity earned

by HIV concerned ELISA and rapid plasma syphilis test. The patient preference for the dongle was 97% as compared to conventional laboratory tests, with most patients acknowledging the rapid results obtained with a single finger-prick as an advantage of the device.

In another study, Jiang et al. (2003) designed a microchip with polyethylene microchannels to detect anti-virus antibodies in the blood. A PDMS microfluidic system comprises polycarbonate membranes forming a micro-dilutor. The micro-dilutor contains chaotic sequential devices for mixing of biofluids and complementary mixing by buffers afterwards. Anti-HIV antibodies from patient blood flow through consecutive mixing and channels across orthogonal, parallel strips bearing immobilized HIV ENV proteins (antigens gp41, and pg120), adsorbed on a poly carbonate membrane. The soluble antibodies bound to adsorbed antigens were measured using a secondary fluorescent antibody. Wang et al. (2010) from Harvard Medical School reported another HIV rapid detection technique using a microfluidic device. HIV-1 particles from 10 μL whole blood were captured by anti-gp120 antibodies coated on the surface of the microchannels and detected by dual fluorescence signals under microscopy. An essential step in the development of this technology to monitor viral load as a POC device, was to establish an automated counting protocol using ImageJ to measure the polystyrene nano-beads. Fig. 5 shows a scheme illustrating a rapid diagnostic microfluidic chip for the detection of HIV-1 viruses.

8.2. HBV detection

HBV (Hepatitis B virus), a double stranded DNA virus that belongs to the genus *Orthohepadnavirus,* was discovered in 1966. HBV is the one of the main causative agents for chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (Lee, 1997). HBV is an epidemic in China with more than 93 million HBV carriers and 20 million people suffering from chronic hepatitis B (Lee, 1997; Lu et al., 2010). Different DNA-based methods can be implemented to detect HBV and these include direct sequencing reduction of fragment polymorphism (Chen et al., 2013), type-specific PCR (Chen et al., 2007) and real-time PCR (Yeh et al., 2004). In one study, Zhi et al. (2014) investigated the detection of HVB using a microfluidic chip. A “Loop Mediated Isothermal Amplification” (LAMP) system, line probe assay (LiPA), and giant magneto-resistive (GMR) sensors were the main components of the apparatus. In this technique, LAMP primers and genotype-specific probes were designed, and “magnetic nanolens particles” (123 nm diameter) synthesized. The microfluidic chip was made of PDMS covered with oligonucleotide probes (target HBV DNA fragments) produced by LAMP and labeled with biotin. Streptavidin conjugated magnetic nanoclusters (5 μg/μL) were injected into the micro-channels and detection occurred through the GMR sensors. The threshold of this detection method is about 10 copies/mL of target HBV DNA in 1 h. In another study, Zhang et al. (2010) used another microfluidic device to detect HBV virus. In this study, a microfluidic chip (lab-on-a-chip type) was combined with modified micro-bead array conjugated with? the Q-dots to detect HBV via DNA segments. A bilayer PDMS microfluidic device was designed and used as a reactor with micro-channels for fluidic stream. The advantages of the technique were the rapid binding kinetics of homogeneous micro-bead assays, liquid handling capability of microfluidics and the fluorescent sensitivity of QDs as well. The device could detect 1000

copies/mL of HBV virus in serum samples (4 pM, S/N>3) using in vitro transcribed RNA as the probe molecules.

8.3. Detection of Zika virus

Zika virus, a member of the Flaviviridae family, was isolated in 1947 from a rhesus macaque monkey in the “Zika Forest” of Uganda, near Lake Victoria (Cohen, 2016) by scientists of the Yellow Fever Research Institute (a) (Malone et al., 2016). According to the global statistics in 2016, Zika virus had been detected in more than 26 countries (Petersen et al., 2016). The virus is transmitted to humans through the bite of mosquitos, *Aedes aegypti* and *Aedes albopictus* (Petersen et al., 2016). The virus can be then passed from an infected to a healthy host by mosquito feeding on both individuals in turn. The infection by Zika virus can be either severe or mild in nature; one of the main sequelae of this infection relates to the risk of microcephaly occurrence in children born from women infected during pregnancy. Currently, there are few highly effective sensors for Zika detection and monitoring. The new generation of portable microfluidic systems helped in detection protocols of ZIKA virus. A wide range of studies aimed at detecting Zika virus have been conducted by different researchers. A report from the laboratory of JJ Collins virus (Pardee et al., 2016) has presented a substrate-based microfluidic system linking isothermal RNA amplification with RNA sensors (Green et al., 2014). In one day, the validated sensors were embedded into paper substrate and were then freeze-dried along. The cell-free transcription and translation system can be deployed in the detecting test. The extracted RNA was isothermally amplified via NASBA and used to rehydrate the freeze-dried paper sensors. The detection of the suitable trigger RNA was indicated by a color shift in the substrate disc from yellow to purple. When coupled with a novel CRISPR/Cas9-based module, the sensors could discriminate between viral strands with single-base resolution. The system was validated by detecting Zika virus in the plasma of an infected macaque (Fig. 6).

Another study from the University of Pennsylvania reported the detection of ZIKA virus using a microfluidic system (Song et al., 2016). A schematic illustration of this microfluidic chip is provided in Fig. 7. The Authors used a highly sensitive reverse-transcription loop-mediated, isothermal amplification (RT-LAMP) assay for rapid detection of ZIKA virus in a simple, easy-to-use, inexpensive, POC disposable cassette. For thermal control of the cassette, a chemically heated cup with no need for electrical power was used. The amplification products were visualized by leuco-crystal violet (LCV) dye, with no need for instrumentation. ZIKA virus detection from oral samples occurred with a sensitivity of 5 plaque-forming units (PFU) in less than 40 min.

9. Future perspectives

Fast, reliable and user-friendly techniques as well as commercially available portable devices with high sensitivity and specificity are required for medical diagnostic purposes (Chin et al., 2012). These requirements become necessary when a dangerous infectious disease threatens to become an epidemic. Progress in the fields of novel diagnostics and therapeutics, especially in the case of lethal viruses like Ebola and Zika, is needed; further,

new detection methods of chronic diseases leading to disability and mortality is also required (Wu et al., 2018).

Despite the fact that these lethal diseases cause death in fewer people than tuberculosis and malaria do even nowadays; this does not diminish public anxiety. Conventional microscopy techniques are currently used for the measurement of erythrocyte deformability, this being particularly relevant to malaria (Shelby et al., 2003; Lee et al., 2007). Sensitive dyes have been developed to enhance optical detection methods such as absorbance, fluorescence (Steingart et al., 2006), confocal microscopy (Psaltis et al., 2007), chemiluminescence (Wang et al., 2012a), dark field microscopy (Hänscheid, 1999), Surface Enhanced Raman Spectroscopy (SERS) (Shanmukh et al., 2008) etc.

When optical detection is combined with microfluidic systems it is known as “optofluidic technology” (Psaltis et al., 2006). To overcome the limitations of optical techniques, CCD-based camera platforms and so-called “lens less imaging” have recently been developed (Moon et al., 2009).

Taton et al. (2000) analyzed combinatorial DNA arrays using oligonucleotide-modified gold nanoparticle probes and a conventional flatbed scanner. The labeling of oligonucleotide targets with nanoparticles rather than fluorophore probes substantially modified the melting profiles of the targets on an array substrate. This difference enabled the identification of an oligonucleotide sequence with a single nucleotide mismatch with sensitivity more than three times higher than fluorophore-labeled targets. When coupled with a signal amplification method based on nanoparticle-promoted reduction of silver salts, the sensitivity exceeded that of the fluorescence system by two orders of magnitude.

The application of nanoparticles conjugated with specific Raman reporter dyes is shown in Fig. 8. In related studies, a Raman reporter or fluorescent dye was used to identify Surface Enhanced Raman Spectroscopy (SERS) peaks with QDts or gold nanoparticles combination (Salmanogli et al., 2017). SERS has also been used because of its high sensitivity at single molecule-level and because of molecular specificity and resistance to quenching (Bantz et al., 2011). SERS has been deployed for evaluation of therapeutic mechanisms, tracing of biomolecules, pathogen identification, cell studies etc. It was also proposed by SalmanOgli et al. to promote mutual near field plasmonic transaction of gold nanoparticles (Yoo and Lee, 2016; SalmanOgli et al., 2017b). The use of gold-based nanoparticles attached to Raman reporter dyes such as rhodamine 6G (Rh6G), rhodamine 123 and 3,3 diethylthiadicarbocyanine iodide (TCDC) enables detection even at very low concentrations in biological fluids (SalmanOgli et al., 2017a) (Fig. 8). Using linkers as spacer arms on nanostructures allows the attachment of a wide range of therapeutic or imaging cargos (SalmanOgli et al., 2016). This approach has been used for nanoparticle-based optical sensors in the detection of pathogenic bacteria (Mocan et al., 2017).

A recent innovation relies on the use of smartphones as image and data acquisition and processing systems for POC and microfluidic devices (Granot et al., 2008; Breslauer et al., 2009). This is based on the remarkable growth of smart-phone usage worldwide, including less-developed regions. In this technique, data were extracted in the form of signal after

sample collection and were subsequently monitored after processing. SERS spectroscopy is included in this category as a portable diagnostic technique.

Priye et al. (2017) used reverse-transcription loop-mediated isothermal amplification (RT-LAMP) coupled with quenching of unincorporated amplification signal reporters (QUASR) technique. These authors used primers for ZIKA, dengue, and chikungunya viruses, all belonging to the flaviviridae family. Reactions were conducted in a simple, inexpensive and portable “LAMP box” fitted within a smartphone. The entire assembly was powered by a 5 V USB source. A novel algorithm was employed to analyze the fluorescence signals, improving the discrimination between positive and negative signals by 5-fold, when compared to naked eye. ZIKA virus could be detected directly from crude human samples (blood, urine, and saliva).

Another novel approach is represented by a nanophotonic lab-on-a-chip platform. In this method, the different biosensing regions were arranged on the surface of the chip. Bimodal waveguide interferometers were fabricated by standard silicon processes and integrated with sub-micron grating couplers for efficient light coupling. A 3D network of polymer microfluidics was monolithically assembled at the wafer-level ensuring perfect sealing and compact assembling. A novel all-optical wavelength modulation system was implemented, providing a linear response and a direct read-out of the phase variation (Fig. 9). The wavelength modulated BiMW sensor was used for the label-free immunodetection of human thyroid stimulating hormone (hTSH). The limit of detection was 3.3×10^{-7} refractive index units, equal to 20 pM of hTSH.

Another novel tool in POC detection utilizes gases as the source of sample in a so-called “electronic nose” (e-nose). An e-nose identifies the specific components of a gas and analyzes its chemical composition, this enabling its identification. An e-nose contains a mechanism for chemical detection, such as an array of electronic sensors and a mechanism for pattern recognition, such as a neural network (Fitzgerald et al., 2017). In medicine, these devices would be most likely used to analyze the exhaled breath. Biomarkers in exhaled breath can be attributed to different diseases caused by bacteria or viruses (Rock et al., 2008). The results obtained after signal processing can be extracted. Patient specific metabolic biomarkers can be then presented as fingerprint results. The e-nose could be therefore used to detect organ malfunction, inflammation in lung disease, biological attacks and bioterrorism etc. (Wilson, 2016).

10. Conclusions

In the present review, we have discussed and compared cell-based protocols and next generation techniques for the diagnosis of pathogenic microorgansims. The microfluidic systems constitute cell free diagnostic tools that can be integrated with other techniques to track and monitor pathogens. As the reported LOC and microarrays are highly variable, we have concentrated on more recent versions of these bioassay platforms. In LOC arrays, thousands of reactions designed to link reagents to particular pathogens or microorganisms can be implemented in a few minutes. The advantages of these high-tech bioassays include the low cost of small quantities of reagents, ease and low cost, as well as the short time

required to obtain the results. The recently developed microfluidic systems constitute rapid detection techniques that do not require the presence of expert operators. Due to these advantages, they represent valuable applications in point of care detection of infectious pathogens. The advantages of microfluidics in the case of small sample volume, diagnosis proficiency and concentration of microorganism within the specimen have been compared with simple multistage detection technologies which may become outdated. Conventional techniques require laborious preparation, isolation, translation and counting steps; furthermore they are time consuming and require high costs. In this review we have also covered DNA based detection such as the PCR protocol. In addition, different modalities of microfluidic diagnostic technologies for the detection of individual microorganisms have been discussed. The different microfluidic chips for the rapid diagnosis of specific pathogens were presented in a table format and their advantages highlighted when compared with conventional protocols. Different modalities of microorganism detection devices such as optic based identification, biosensing, colorimetric methods and nanophotonics used in microfluidics were discussed. The recent developments in the field of microarray systems (microfluidic chips for diagnosis, POC and LOC) play a main role. The large amount of biological results it is possible to obtain using these techniques can constitute a formidable set of data that requires dedicated analysis software. The integration of PCR and microarray RT-PCR (Real-Time PCR Array) for the diagnosis of various samples is a valuable addition. In the future perspectives section we have also reported studies where the combination of microfluidic chips and optoelectronic principal platforms have been attempted. Amongst the novel techniques reported in these studies, the enhancement of optical waves has been achieved through the use of noble metals such as gold and fluorescence dyes or Raman reporters conjugated with nanoparticles. The light scattered from the surface of the microarray platform transfers valuable information about the microorganisms to a smart-phone device to further evaluate and monitor the results. In this smart-phone supported diagnostic apparatus, the specific antibodies were conjugated onto nanoparticles in the microfluidic chip or microarray.

Protein based pathogen capture systems are commonly used in microfluidic chips. The recently developed microfluidic chips for POC diagnosis and the multi-detection microarrays without cell culturing steps, offer numerous advantages when compared to conventional techniques that, instead, require cell culture steps. The advantages include high accuracy, easy use, shorter time for obtaining results and lower costs.

In summary, a large number of studies on microfluidic systems used for the detection of microorganisms have been published in high impact journals. Furthermore, recent developments in the application of microfluidic systems can help human health in under-developed regions with poor health infrastructure. These techniques should be considered as the most promising detection methods for the future due to their rapid implementation, easy applicability and lower costs.

References

Abadian PN, Kelley CP, Goluch ED. Cellular analysis and detection using surface plasmon resonance techniques. Anal Chem. 2014; 86(6):2799–2812. [PubMed: 24502446]

Anderson JR, et al. Fabrication of microfluidic systems in poly (dimethylsiloxane). Electrophoresis. 2000; 21(1):27–40. [PubMed: 10634468]

Bantz KC, et al. Recent progress in SERS biosensing. Phys Chem Chem Phys. 2011; 13(24):11551–11567. [PubMed: 21509385]

Batalla P, et al. Enzyme-based microfluidic chip coupled to graphene electrodes for the detection of D-amino acid enantiomer-biomarkers. Anal Chem. 2015; 87(10):5074–5078. [PubMed: 25870911]

Bean CP, Livingston JD. Superparamagnetism. J Appl Phys. 1959; 30:S120.

Becker H, Gärtner C. Polymer microfabrication technologies for microfluidic systems. Anal Bioanal Chem. 2008; 390(1):89–111. [PubMed: 17989961]

Beebe DJ, et al. Microfluidic tectonics: a comprehensive construction platform for microfluidic systems. Proc Natl Acad Sci USA. 2000; 97(25):13488–13493. [PubMed: 11087831]

Boehm DA, Gottlieb PA, Hua SZ. On-chip microfluidic biosensor for bacterial detection and identification. Sens Actuators B: Chem. 2007; 126(2):508–514.

Bonifacio A, Cervo S, Sergo V. Label-free surface-enhanced Raman spectroscopy of biofluids: fundamental aspects and diagnostic applications. Anal Bioanal Chem. 2015; 407(27):8265–8277. [PubMed: 25935674]

Breslauer DN, et al. Mobile phone based clinical microscopy for global health applications. PLoS One. 2009; 4(7):e6320. [PubMed: 19623251]

Bruno JG. Application of DNA aptamers and quantum dots to lateral flow test strips for detection of foodborne pathogens with improved sensitivity versus colloidal gold. Pathogens. 2014; 3(2):341–355. [PubMed: 25437803]

Carey JR, et al. Rapid identification of bacteria with a disposable colorimetric sensing array. J Am Chem Soc. 2011; 133(19):7571–7576. [PubMed: 21524080]

Chen J, et al. Improved multiplex-PCR to identify hepatitis B virus genotypes A–F and subgenotypes B1, B2, C1 and C2. J Clin Virol. 2007; 38(3):238–243. [PubMed: 17224304]

Chen L, Choo J. Recent advances in surface-enhanced Raman scattering detection technology for microfluidic chips. Electrophoresis. 2008; 29(9):1815–1828. [PubMed: 18384070]

Chen Y, et al. Hepatitis B virus subgenotype C2- and B2-associated mutation patterns may be responsible for liver cirrhosis and hepatocellular carcinoma, respectively. Braz J Med Biol Res. 2013; 46(7):614–622. [PubMed: 23903686]

Cheng I-F, et al. A dielectrophoretic chip with a roughened metal surface for on-chip surface-enhanced Raman scattering analysis of bacteria. Biomicrofluidics. 2010; 4(3):034104. [PubMed: 20806000]

Chiem N, Harrison DJ. Microchip-based capillary electrophoresis for immunoassays: analysis of monoclonal antibodies and theophylline. Anal Chem. 1997; 69(3):373–378. [PubMed: 9030052]

Chin CD, et al. Microfluidics-based diagnostics of infectious diseases in the developing world. Nat Med. 2011; 17(8):1015–1019. [PubMed: 21804541]

Chin CD, Linder V, Sia SK. Lab-on-a-chip devices for global health: past studies and future opportunities. Lab a Chip. 2007; 7(1):41–57.

Chin CD, Linder V, Sia SK. Commercialization of microfluidic point-of-care diagnostic devices. Lab Chip. 2012; 12(12):2118–2134. [PubMed: 22344520]

Chiu DT, et al. Small but perfectly formed? Successes, challenges, and opportunities for microfluidics in the chemical and biological sciences. Chem. 2017; 2(2):201–223.

Chung HJ, et al. A magneto-DNA nanoparticle system for rapid detection and phenotyping of bacteria. Nat Nanotechnol. 2013; 8(5):369–375. [PubMed: 23644570]

Cohen J. Zika's long, strange trip into the limelight. Science. 2016

De La Rica R, Stevens MM. Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nat Nanotechnol. 2012; 7(12):821. [PubMed: 23103935]

Diamandis EP. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool opportunities and potential limitations. Mol Cell Proteom. 2004; 3(4):367–378.

Dina N, et al. Rapid single-cell detection and identification of pathogens by using surface-enhanced Raman spectroscopy. Analyst. 2017; 142(10):1782–1789. [PubMed: 28430277]

Dittrich PS, Tachikawa K, Manz A. Micro total analysis systems. Latest advancements and trends. Anal Chem. 2006; 78(12):3887–3908. [PubMed: 16771530]

Duffy DC, et al. Rapid prototyping of microfluidic systems in poly (dimethylsiloxane). Anal Chem. 1998; 70(23):4974–4984. [PubMed: 21644679]

Duval D, et al. Nanophotonic lab-on-a-chip platforms including novel bimodal interferometers, microfluidics and grating couplers. Lab Chip. 2012; 12(11):1987–1994. [PubMed: 22538502]

Ertl P, et al. Capillary electrophoresis chips with a sheath-flow supported electrochemical detection system. Anal Chem. 2004; 76(13):3749–3755. [PubMed: 15228350]

Fire A, Xu S-Q. Rolling replication of short DNA circles. Proc Natl Acad Sci USA. 1995; 92(10): 4641–4645. [PubMed: 7753856]

Fiscus SA, et al. HIV-1 viral load assays for resource-limited settings. PLoS Med. 2006; 3(10):e417. [PubMed: 17032062]

Fitzgerald JE, et al. Artificial nose technology: status and prospects in diagnostics. Trends Biotechnol. 2017; 35(1):33–42. [PubMed: 27612567]

Foudeh AM, et al. Microfluidic designs and techniques using lab-on-a-chip devices for pathogen detection for point-of-care diagnostics. Lab Chip. 2012; 12(18):3249–3266. [PubMed: 22859057]

Funes-Huacca M, et al. Portable self-contained cultures for phage and bacteria made of paper and tape. Lab Chip. 2012; 12(21):4269–4278. [PubMed: 22895550]

Fykse EM, et al. Detection of Vibrio cholerae by real-time nucleic acid sequence-based amplification. Appl Environ Microbiol. 2007; 73(5):1457–1466. [PubMed: 17220262]

Gaits F, Hahn K. Shedding light on cell signaling: interpretation of FRET biosensors. Sci STKE. 2003; 2003(165):pe3–pe3. [PubMed: 12527820]

Gau J-J, et al. A MEMS based amperometric detector for E. coli bacteria using self-assembled monolayers. Biosens Bioelectron. 2001; 16(9):745–755. [PubMed: 11679252]

Gervais L, De Rooij N, Delamarche E. Microfluidic chips for point-of-care immunodiagnostics. Adv Mater. 2011; 23(24)

Ghazani AA, et al. Comparison of select cancer biomarkers in human circulating and bulk tumor cells using magnetic nanoparticles and a miniaturized micro-NMR system. Nanomedicine. 2013; 9(7): 1009–1017. [PubMed: 23570873]

DbER GOB, et al. Direct quantification of analyte concentration by resonant acoustic profiling. Clin Chem. 2005; 51(10):1962–1972. [PubMed: 16081504]

Gomez-Sjoberg R, Morisette DT, Bashir R. Impedance microbiology-on-a-chip: microfluidic bioprocessor for rapid detection of bacterial metabolism. J Micro Syst. 2005; 14(4):829–838.

Granot Y, Ivorra A, Rubinsky B. A new concept for medical imaging centered on cellular phone technology. PLoS One. 2008; 3(4):e2075. [PubMed: 18446199]

Green AA, et al. Toehold switches: de-novo-designed regulators of gene expression. Cell. 2014; 159(4):925–939. [PubMed: 25417166]

Guan X, et al. Rapid detection of pathogens using antibody-coated microbeads with bioluminescence in microfluidic chips. Biomed Microevices. 2010; 12(4):683–691.

Hänscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy. Int J Lab Hematol. 1999; 21(4):235–245.

Harrison DJ, et al. Capillary electrophoresis and sample injection systems integrated on a planar glass chip. Anal Chem. 1992; 64(17):1926–1932.

Hart R, et al. Point-of-care oral-based diagnostics. Oral Dis. 2011; 17(8):745–752. [PubMed: 21521419]

Haun JB, et al. Micro-NMR for rapid molecular analysis of human tumor samples. Sci Transl Med. 2011; 3(71):71ra16–71ra16.

HIV/AIDS JUNPo. Ending AIDS: Progress Towards the 90-90-90 Targets. Global AIDS Update. 2017

Hochstetler S, Matosky A. Detection of living cells in polymers or pigments. Google Patents. 2005

Hoppmann EP, Wei WY, White IM. Inkjet-printed fluidic paper devices for chemical and biological analytics using surface enhanced Raman spectroscopy. IEEE J Sel Top Quantum Electron. 2014; 20(3):195–204.

Hossain SZ, et al. Multiplexed paper test strip for quantitative bacterial detection. Anal Bioanal Chem. 2012; 403(6):1567–1576. [PubMed: 22526653]

Huang G, et al. A rapid, low-cost, and microfluidic chip-based system for parallel identification of multiple pathogens related to clinical pneumonia. Sci Rep. 2017; 7(1):6441. [PubMed: 28743917]

Huq A, Colwell RR. A microbiological paradox: viable but not culturable bacteria with special reference to Vibrio cholerae. J Food Prot. 1996; 59:96–101.

Ionescu-Zanetti C, et al. Mammalian electrophysiology on a microfluidic platform. Proc Natl Acad Sci USA. 2005; 102(26):9112–9117. [PubMed: 15967996]

Issadore D, et al. Miniature magnetic resonance system for point-of-care diagnostics. Lab Chip. 2011; 11(13):2282–2287. [PubMed: 21547317]

Janasek D, Franzke J, Manz A. Scaling and the design of miniaturized chemical-analysis systems. Nature. 2006; 442(7101):374. [PubMed: 16871204]

Jarvius J, et al. Digital quantification using amplified single-molecule detection. Nat Methods. 2006; 3(9):725–727. [PubMed: 16929318]

Jayamohan H, et al. Advances in Microfluidics and Lab-on-a-Chip Technologies. 2017 arXiv preprint ⟨arXiv:1703.08583⟩.

Jiang X, et al. A miniaturized, parallel, serially diluted immunoassay for analyzing multiple antigens. J Am Chem Soc. 2003; 125(18):5294–5295. [PubMed: 12720439]

Jokerst JC, et al. Development of a paper-based analytical device for colorimetric detection of select foodborne pathogens. Anal Chem. 2012; 84(6):2900–2907. [PubMed: 22320200]

Joung H-A, Oh YK, Kim M-G. An automatic enzyme immunoassay based on a chemiluminescent lateral flow immunosensor. Biosens Bioelectron. 2014; 53:330–335. [PubMed: 24176968]

Kang T, et al. Patterned multiplex pathogen DNA detection by Au particle-on-wire SERS sensor. Nano Lett. 2010; 10(4):1189–1193. [PubMed: 20222740]

Kaur H, Dhanao J, Oberoi A. Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. Indian J Med Sci. 2000; 54(10):432–434. [PubMed: 11262859]

Ke C, et al. Rapid amplification for the detection of Mycobacterium tuberculosis using a non-contact heating method in a silicon microreactor based thermal cycler. Sens Actuators B: Chem. 2004; 102(2):308–314.

Kim D-K, et al. Plasmonic properties of the multispot copper-capped nanoparticle array chip and its application to optical biosensors for pathogen detection of multiplex DNAs. Anal Chem. 2011; 83(16):6215–6222. [PubMed: 21714496]

Kim E, Xia Y, Whitesides GM. Micromolding in capillaries: applications in materials science. J Am Chem Soc. 1996; 118:5722–5731.

Kim G, et al. A microfluidic nano-biosensor for the detection of pathogenic Salmonella. Biosens Bioelectron. 2015; 67:243–247. [PubMed: 25172028]

Ko H, et al. Active digital microfluidic paper chips with inkjet-printed patterned electrodes. Adv Mater. 2014; 26(15):2335–2340. [PubMed: 24729060]

Koch WH, et al. Rapid polymerase chain reaction method for detection of Vibrio cholerae in foods. Appl Environ Microbiol. 1993; 59(2):556–560. [PubMed: 8434922]

Kong R, et al. Rapid detection of six types of bacterial pathogens in marine waters by multiplex PCR. Water Res. 2002; 36(11):2802–2812. [PubMed: 12146868]

Kopp MU, De Mello AJ, Manz A. Chemical amplification: continuous-flow PCR on a chip. Science. 1998; 280(5366):1046–1048. [PubMed: 9582111]

Kramer MF, Lim DV. A rapid and automated fiber optic–based biosensor assay for the detection of Salmonella in spent irrigation water used in the sprouting of sprout seeds. J Food Prot. 2004; 67(1):46–52. [PubMed: 14717350]

Lagally ET, Simpson PC, Mathies RA. Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system. Sens Actuators B: Chem. 2000; 63(3):138–146.

Laksanasopin T, et al. A smartphone dongle for diagnosis of infectious diseases at the point of care. Sci Transl Med. 2015; 7(273):273re1–273re1.

Lee H, et al. Chip–NMR biosensor for detection and molecular analysis of cells. Nat Med. 2008; 14(8):869–874. [PubMed: 18607350]

Lee H, Yoon TJ, Weissleder R. Ultrasensitive detection of bacteria using core-shell nanoparticles and an NMR-filter system. Angew Chem Int Ed Engl. 2009; 48(31):5657–5660. [PubMed: 19554581]

Lee SJ, et al. A microfluidic ATP-bioluminescence sensor for the detection of airborne microbes. Sens Actuators B: Chem. 2008; 132(2):443–448.

Lee WG, et al. On-chip erythrocyte deformability test under optical pressure. Lab Chip. 2007; 7(4): 516–519. [PubMed: 17389970]

Lee WM. Medical progress. N Engl J Med. 1997; 337:1733–1745. [PubMed: 9392700]

Lei KF. Microfluidic systems for diagnostic applications: a review. J Lab Autom. 2012; 17(5):330–347. [PubMed: 22893635]

Li PC, Harrison DJ. Transport, manipulation, and reaction of biological cells on-chip using electrokinetic effects. Anal Chem. 1997; 69(8):1564–1568. [PubMed: 9109354]

Li Y, SUXL. Microfluidics-based optical biosensing method for rapid detection of Escherichia coli O157: H7. J Rapid Methods Autom Microbiol. 2006; 14(1):96–109.

Liong M, et al. Magnetic barcode assay for genetic detection of pathogens. Nat Commun. 2013; 4:1752. [PubMed: 23612293]

Liu GL, Lee LP. Nanowell surface enhanced Raman scattering arrays fabricated by soft-lithography for label-free biomolecular detections in integrated microfluidics. Appl Phys Lett. 2005; 87(7): 074101.

Liu RH, et al. Integrated microfluidic CustomArray device for bacterial genotyping and identification. JALA: J Assoc Lab Autom. 2006; 11:360–367.

Liu RH, et al. Validation of a fully integrated microfluidic array device for influenza A subtype identification and sequencing. Anal Chem. 2006; 78(12):4184–4193. [PubMed: 16771549]

Liu S, et al. Optimization of high-speed DNA sequencing on microfabricated capillary electrophoresis channels. Anal Chem. 1999; 71(3):566–573. [PubMed: 9989377]

Lu F, et al. Epidemiology and prevention of hepatitis B virus infection in China. J Viral Hepat. 2010; 17(s1):4–9. [PubMed: 20586928]

Malic L, Veres T, Tabrizian M. Biochip functionalization using electrowetting-on-dielectric digital microfluidics for surface plasmon resonance imaging detection of DNA hybridization. Biosens Bioelectron. 2009; 24(7):2218–2224. [PubMed: 19136248]

Malone RW, et al. Zika virus: medical countermeasure development challenges. PLoS Negl Trop Dis. 2016; 10(3):e0004530. [PubMed: 26934531]

Manganelli R, et al. Differential expression of 10 sigma factor genes in Mycobacterium tuberculosis. Mol Microbiol. 1999; 31(2):715–724. [PubMed: 10027986]

Manz A, Becker H. Parallel capillaries for high throughput in electrophoretic separations and electroosmotic drug discovery systems. Solid State Sensors and Actuators; 1997; TRANSDUCERS'97 Chicago, 1997 International Conference on; IEEE; 1997.

Mason H-Y, et al. Taxonomic identification of microorganisms by capture and intrinsic fluorescence detection. Biosens Bioelectron. 2003; 18(5):521–527. [PubMed: 12706558]

McNerney R, Daley P. Towards a point-of-care test for active tuberculosis: obstacles and opportunities. Nat Rev Microbiol. 2011; 9(3):204. [PubMed: 21326275]

Mocan T, et al. Development of nanoparticle-based optical sensors for pathogenic bacterial detection. J Nanobiotechnol. 2017; 15(1):25.

Moller-Kristensen M, et al. Burn injury reveals altered phenotype in mannan-binding lectin-deficient mice. J Invest Dermatol. 2007; 127(6):1524–1531. [PubMed: 17363917]

Moon S, et al. Integrating microfluidics and lensless imaging for point-of-care testing. Biosens Bioelectron. 2009; 24(11):3208–3214. [PubMed: 19467854]

Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. Nature. 2004; 430(6996):242–249. [PubMed: 15241422]

Mujika M, et al. Magnetoresistive immunosensor for the detection of Escherichia coli O157: H7 including a microfluidic network. Biosens Bioelectron. 2009; 24(5):1253–1258. [PubMed: 18760584]

Mukhopadhyay R. When PDMS isn't the Best ACS Publications. 2007.

Muñoz-Elías EJ, et al. Replication dynamics of Mycobacterium tuberculosis in chronically infected mice. Infect Immun. 2005; 73(1):546–551. [PubMed: 15618194]

Myers K, Gaba J, Al-Khaldi S. Molecular identification of Yersinia enterocolitica isolated from pasteurized whole milk using DNA microarray chip hybridization. Mol Cell Probes. 2006; 20(2): 71–80. [PubMed: 16330182]

Na W, et al. Rapid molecular diagnosis of infectious viruses in microfluidics using DNA hydrogel formation. Biosens Bioelectron. 2018; 108:9–13. [PubMed: 29494886]

Navarro-Noya Y, et al. 16S rRNA gene-based identification of bacteria in postoperative endophthalmitis by PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) fingerprinting. Braz J Microbiol. 2012; 43(1):283–287. [PubMed: 24031830]

O'Sullivan CE, et al. Evaluation of Gen-Probe amplified Mycobacterium tuberculosis direct test by using respiratory and nonrespiratory specimens in a tertiary care center laboratory. J Clin Microbiol. 2002; 40(5):1723–1727. [PubMed: 11980950]

Pardee K, et al. Rapid, low-cost detection of Zika virus using programmable biomolecular components. Cell. 2016; 165(5):1255–1266. [PubMed: 27160350]

Park S, et al. Advances in microfluidic PCR for point-of-care infectious disease diagnostics. Biotechnol Adv. 2011; 29(6):830–839. [PubMed: 21741465]

Peng S, et al. Synthesis and stabilization of monodisperse Fe nanoparticles. J Am Chem Soc. 2006; 128(33):10676–10677. [PubMed: 16910651]

Petersen E, et al. Rapid spread of Zika virus in the Americas-implications for public health preparedness for mass gatherings at the 2016 Brazil Olympic Games. Int J Infect Dis. 2016; 44:11–15. [PubMed: 26854199]

Petersen LR, et al. Zika virus. N Engl J Med. 2016; 374(16):1552–1563. [PubMed: 27028561]

Pöhlmann C, Dieser I, Sprinzl M. A lateral flow assay for identification of Escherichia coli by ribosomal RNA hybridisation. Analyst. 2014; 139(5):1063–1071. [PubMed: 24443718]

Priye A, et al. A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. Sci Rep. 2017:7. [PubMed: 28127057]

Psaltis AJ, et al. Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. Laryngoscope. 2007; 117(7):1302–1306. [PubMed: 17603329]

Psaltis D, Quake SR, Yang C. Developing optofluidic technology through the fusion of microfluidics and optics. Nature. 2006; 442(7101):381–386. [PubMed: 16871205]

Radke SM, Alocilja EC. Design and fabrication of a microimpedance biosensor for bacterial detection. IEEE Sens J. 2004; 4(4):434–440.

Reyes DR, et al. Micro total analysis systems. 1. Introduction, theory, and technology. Anal Chem. 2002; 74(12):2623–2636. [PubMed: 12090653]

Rider TH, et al. AB cell-based sensor for rapid identification of pathogens. Science. 2003; 301(5630): 213–215. [PubMed: 12855808]

Rock F, Barsan N, Weimar U. Electronic nose: current status and future trends. Chem Rev. 2008; 108(2):705–725. [PubMed: 18205411]

Sackmann EK, Fulton AL, Beebe DJ. The present and future role of microfluidics in biomedical research. Nature. 2014; 507(7491):181. [PubMed: 24622198]

Salimi-Moosavi H, Tang T, Harrison DJ. Electroosmotic pumping of organic solvents and reagents in microfabricated reactor chips. J Am Chem Soc. 1997; 119(37):8716–8717.

Salmanogli A, Nasseri B, Pişkin E. Plasmon-plasmon interaction effect on reproducible surface-enhanced Raman scattering for dye molecule detection. Sens Actuators A: Phys. 2017; 262:87–98.

SalmanOgli A, et al. Plasmon - plasmon interaction effect on effective medium electrical conductivity (an effective agent for photothermal therapy). Curr Appl Phys. 2016; 16(11):1498–1505.

SalmanOgli A, Nasseri B, Piskin E. Plasmon–plasmon interaction effect on effective medium electrical conductivity (an effective agent for photothermal therapy). Curr Appl Phys. 2016; 16(11):1498–1505.

SalmanOgli A, Nasseri B, Pişkin E. Plasmon-Plasmon interaction effect on reproducible surface-enhanced Raman scattering for dye molecule detection. Sens Actuators A: Phys. 2017

SalmanOgli A, Nasseri B, Pişkin E. Highly field enhancement by plasmonic field engineering in random distribution of Au-Au nanoparticles as SERS structure. J Lumin. 2017; 190:386–391.

San Park T, et al. Smartphone quantifies Salmonella from paper microfluidics. Lab Chip. 2013; 13(24): 4832–4840. [PubMed: 24162816]

Shanmukh S, et al. Identification and classification of respiratory syncytial virus (RSV) strains by surface-enhanced Raman spectroscopy and multivariate statistical techniques. Anal Bioanal Chem. 2008; 390(6):1551–1555. [PubMed: 18236030]

Shao H, et al. Magnetic nanoparticles and microNMR for diagnostic applications. Theranostics. 2012; 2(1):55–65. [PubMed: 22272219]

Shelby JP, et al. A microfluidic model for single-cell capillary obstruction by Plasmodium falciparum-infected erythrocytes. Proc Natl Acad Sci USA. 2003; 100(25):14618–14622. [PubMed: 14638939]

Silley P, Forsythe S. Impedance microbiology—a rapid change for microbiologists. J Appl Microbiol. 1996; 80(3):233–243.

Simpson PC, et al. High-throughput genetic analysis using microfabricated 96-sample capillary array electrophoresis microplates. Proc Natl Acad Sci USA. 1998; 95(5):2256–2261. [PubMed: 9482872]

Sin ML, et al. Integrated microfluidic systems for molecular diagnostics: a universal electrode platform for rapid diagnosis of urinary tract infections. IEEE Nanotechnol Mag. 2013; 7(1):31–37.

Song J, et al. Instrument-free point-of-care molecular detection of Zika virus. Anal Chem. 2016; 88(14):7289–7294. [PubMed: 27306491]

Song J, et al. Two-stage isothermal enzymatic amplification for concurrent multiplex molecular detection. Clin Chem. 2017; 63(3):714–722. [PubMed: 28073898]

Steingart KR, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. 2006; 6(9):570–581. [PubMed: 16931408]

Stokes DL, Griffin GD, Vo-Dinh T. Detection of E. coli using a microfluidics-based antibody biochip detection system Fresenius'. J Anal Chem. 2001; 369(3):295–301.

Streets AM, Huang Y. Chip in a lab: microfluidics for next generation life science research. Biomicrofluidics. 2013; 7(1):011302.

Tamarin O, et al. Real time device for biosensing: design of a bacteriophage model using love acoustic waves. Biosens Bioelectron. 2003; 18(5):755–763. [PubMed: 12706589]

Tani H, Maehana K, Kamidate T. Chip-based bioassay using bacterial sensor strains immobilized in three-dimensional microfluidic network. Anal Chem. 2004; 76(22):6693–6697. [PubMed: 15538793]

Taton TA, Mirkin CA, Letsinger RL. Scanometric DNA array detection with nanoparticle probes. Science. 2000; 289(5485):1757–1760. [PubMed: 10976070]

Tawil N, et al. Surface plasmon resonance detection of E. coli and methicillin-resistant S. aureus using bacteriophages. Biosens Bioelectron. 2012; 37(1):24–29. [PubMed: 22609555]

Terry SC, Jerman JH, Angell JB. A gas chromatographic air analyzer fabricated on a silicon wafer. IEEE Trans Electron Devices. 1979; 26(12):1880–1886.

Tram K, et al. Translating bacterial detection by DNAzymes into a litmus test. Angew Chem Int Ed. 2014; 53(47):12799–12802.

Varshney M, Li Y. Interdigitated array microelectrode based impedance biosensor coupled with magnetic nanoparticle–antibody conjugates for detection of Escherichia coli O157: H7 in food samples. Biosens Bioelectron. 2007; 22(11):2408–2414. [PubMed: 17045791]

Vilkner T, Janasek D, Manz A. Micro total analysis systems. Recent developments. Anal Chem. 2004; 76(12):3373–3386. [PubMed: 15193114]

Wan Y, et al. Multifunctional semiconducting polymer dots for imaging, detection, and photo-killing of bacteria. J Mater Chem B. 2014; 2(30):4818–4825.

Wang F, et al. Chemiluminescence molecular detection of sequence-specific HBV-DNA using magnetic nanoparticles. J Biomed Nanotechnol. 2012; 8(5):786–790. [PubMed: 22888749]

Wang J-H, , et al. Integrated microfluidic system for HIV detection. In: Micro Electro Mechanical Systems (MEMS); 2012 IEEE 25th International Conference on; IEEE; 2012b.

Wang L, et al. Fungal pathogenic nucleic acid detection achieved with a microfluidic microarray device. Anal Chim Acta. 2008; 610(1):97–104. [PubMed: 18267145]

Wang S, , et al. Proc SPIE(NIH Public Access). 2010. Development of a microfluidic system for measuring HIV-1 viral load.

Wang Y, et al. Application of nanodiagnostics in point-of-care tests for infectious diseases. Int J Nanomed. 2017; 12:4789.

Wang Y, Knoll W, Dostalek J. Bacterial pathogen surface plasmon resonance biosensor advanced by long range surface plasmons and magnetic nanoparticle assays. Anal Chem. 2012; 84(19):8345–8350. [PubMed: 22931462]

Weibel DB, DiLuzio WR, Whitesides GM. Microfabrication meets microbiology. Nat Rev Microbiol. 2007; 5(3):209–218. [PubMed: 17304250]

Whitesides G. The origins and the future of microfluidics. Nature. 2006 4427101 3683730028–3683730836.

Wilson D. Recent progress in the design and clinical development of electronic-nose technologies. Nanobiosens Dis Diagn. 2016

Woolley AT, Mathies RA. Ultra-high-speed DNA sequencing using capillary electrophoresis chips. Anal Chem. 1995; 67(20):3676–3680. [PubMed: 8644919]

Wu J, et al. Lab-on-chip technology for chronic disease diagnosis npj. Digit Med. 2018; 1(1):7.

Xia Y, Whitesides GM. Soft lithography. Annu Rev Mater Sci. 1998; 28(1):153–184.

Xiang Q, et al. Miniaturized immunoassay microfluidic system with electrokinetic control. Biosens Bioelectron. 2006; 21(10)

Yadavalli VK, Pishko MV. Biosensing in microfluidic channels using fluorescence polarization. Anal Chim Acta. 2004; 507(1):123–128.

Yager P, et al. Microfluidic diagnostic technologies for global public health. Nature. 2006; 442(7101): 412–418. [PubMed: 16871209]

Yang M, et al. Highly specific and cost-efficient detection of Salmonella Paratyphi A combining aptamers with single-walled carbon nanotubes. Sensors. 2013; 13(5):6865–6881. [PubMed: 23698275]

Yeh S-H, et al. Quantification and genotyping of hepatitis B virus in a single reaction by real-time PCR and melting curve analysis. J Hepatol. 2004; 41(4):659–666. [PubMed: 15464248]

Yi C, et al. Optical and electrochemical detection techniques for cell-based microfluidic systems. Anal Bioanal Chem. 2006; 384(6):1259–1268. [PubMed: 16795144]

Yildiz FH, Schoolnik GK. Vibrio cholerae O1 El Tor: identification of a gene cluster required for the rugose colony type, exopolysaccharide production, chlorine resistance, and biofilm formation. Proc Natl Acad Sci USA. 1999; 96(7):4028–4033. [PubMed: 10097157]

Yoo SM, et al. DNA microarray-based identification of bacterial and fungal pathogens in bloodstream infections. Mol Cell Probes. 2010; 24(1):44–52. [PubMed: 19818395]

Yoo SM, Lee SY. Optical biosensors for the detection of pathogenic microorganisms. Trends Biotechnol. 2016; 34(1):7–25. [PubMed: 26506111]

Yoo SM, Kim D-K, Lee SY. Aptamer-functionalized localized surface plasmon resonance sensor for the multiplexed detection of different bacterial species. Talanta. 2015; 132:112–117. [PubMed: 25476286]

Zhang H, et al. A microfluidic device with microbead array for sensitive virus detection and genotyping using quantum dots as fluorescence labels. Biosens Bioelectron. 2010; 25(11):2402–2407. [PubMed: 20483585]

Zhi X, et al. A novel HBV genotypes detecting system combined with microfluidic chip, loop-mediated isothermal amplification and GMR sensors. Biosens Bioelectron. 2014; 54:372–377. [PubMed: 24292142]

Zhou J, et al. Convenient formation of nanoparticle aggregates on microfluidic chips for highly sensitive SERS detection of biomolecules. Anal Bioanal Chem. 2012; 402(4):1601–1609. [PubMed: 22127578]

Zourob M, et al. Bacteria detection using disposable optical leaky waveguide sensors. Biosens Bioelectron. 2005; 21(2):293–302. [PubMed: 16023956]

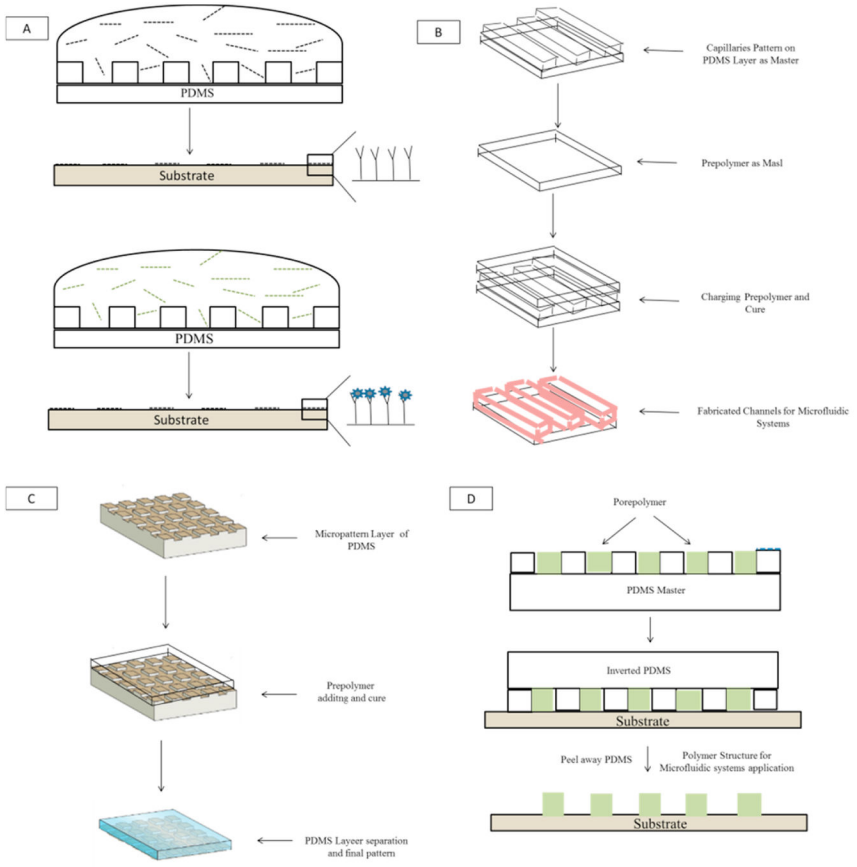

Fig. 1.

Schematic illustration of the different techniques used in micropattern soft lithography (photolithography) to develop microarray templates and design microchannels in microfluidic systems. These techniques include: A) Microcontact printing, B) Micromolding in capillaries, C) Replica molding and D) Microtransfer molding technique.

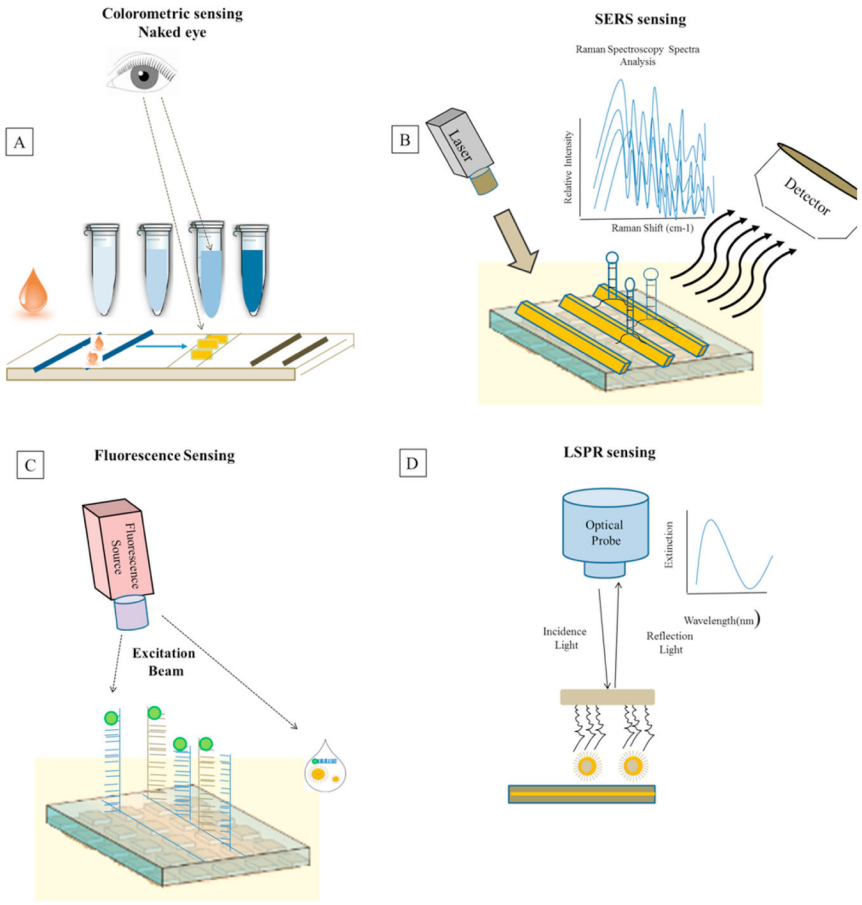

Fig. 2.
Optical techniques used for the detection of microorganisms.

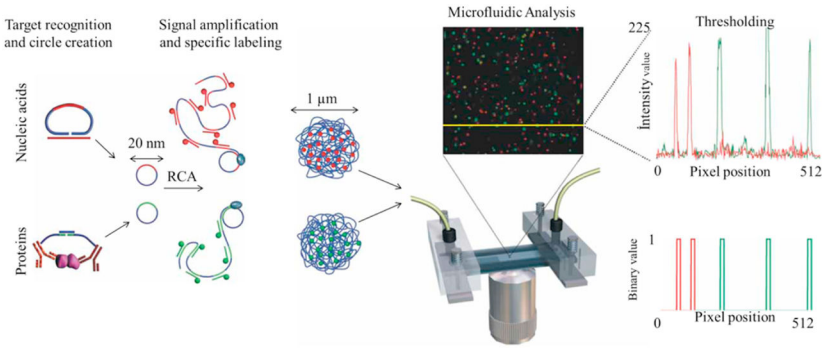

Fig. 3.

Mechanism for rolling circle amplified single-molecule detection (SMD) (Jarvius et al., 2006). Reprinted with permission.

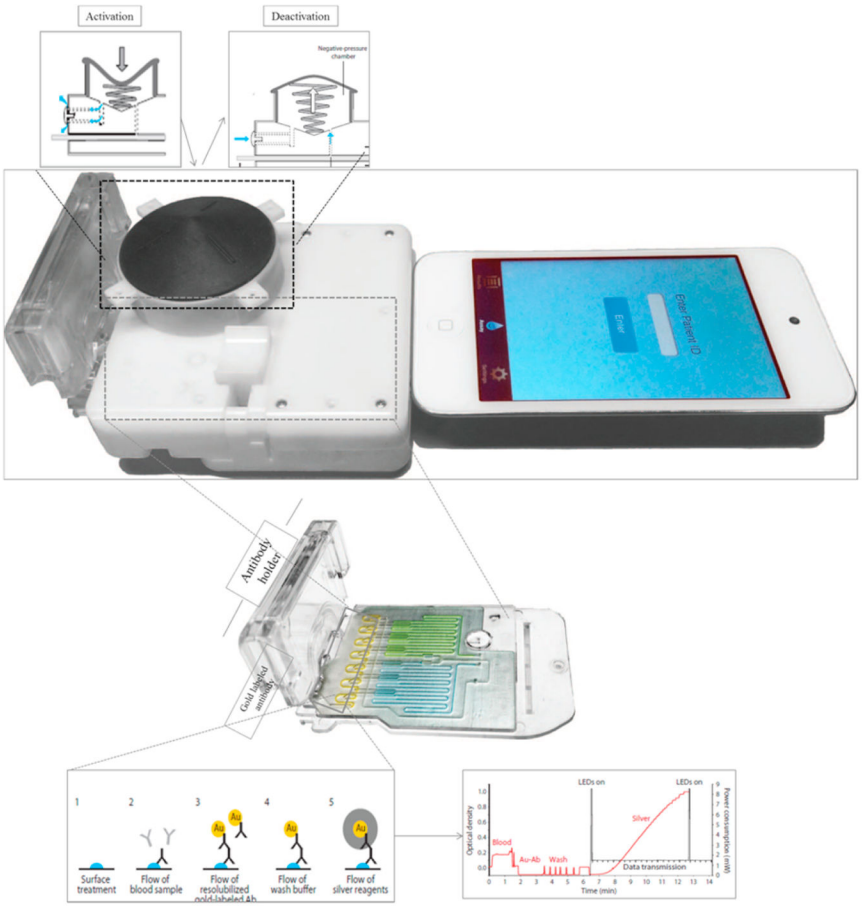

Fig. 4.

A smartphone dongle for the diagnosis of infectious pathogens (Laksanasopin et al., 2015) Reprinted with permission.

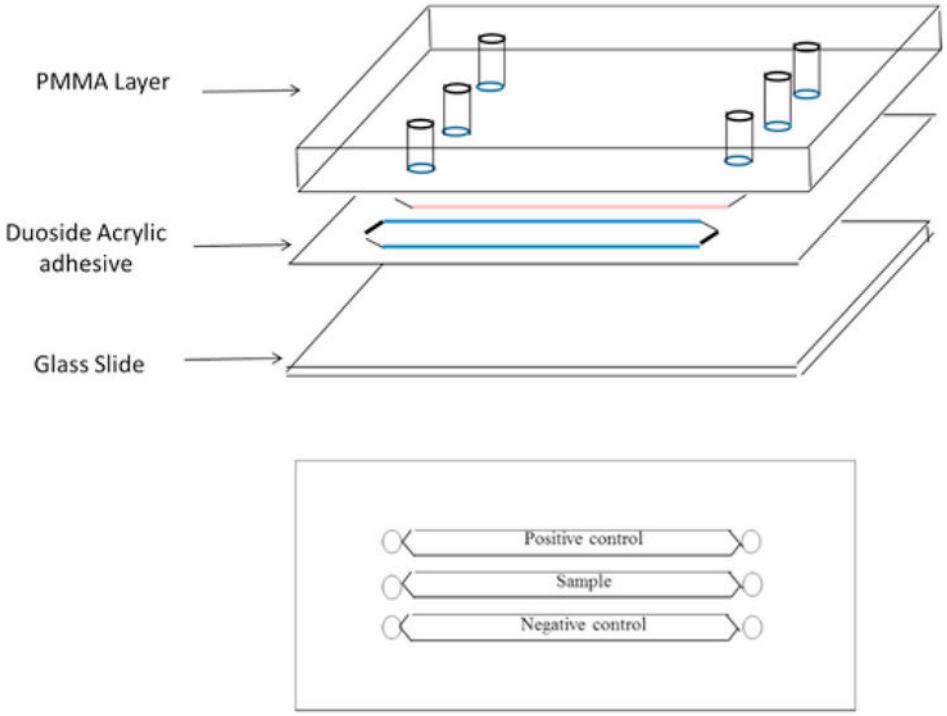

Fig. 5.
Schematic illustration of rapid HIV detection using a microfluidic device.

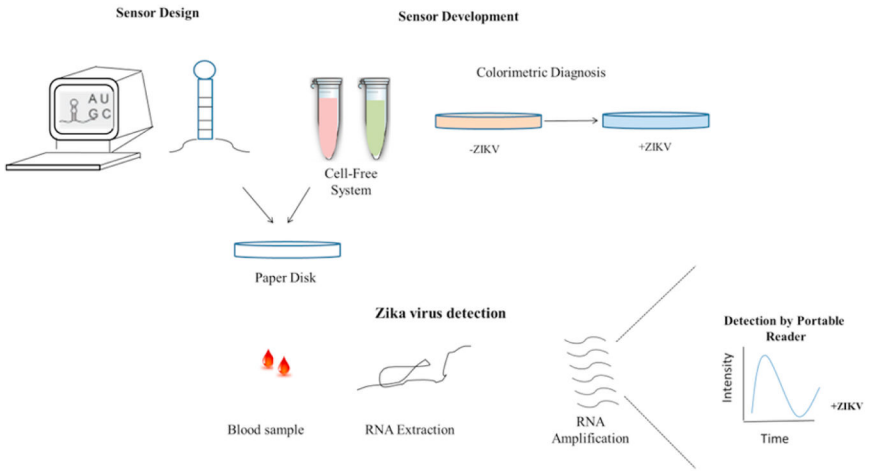

Fig. 6.
Cell-free paper based optical genome sensor, Pardee et al. (2016).

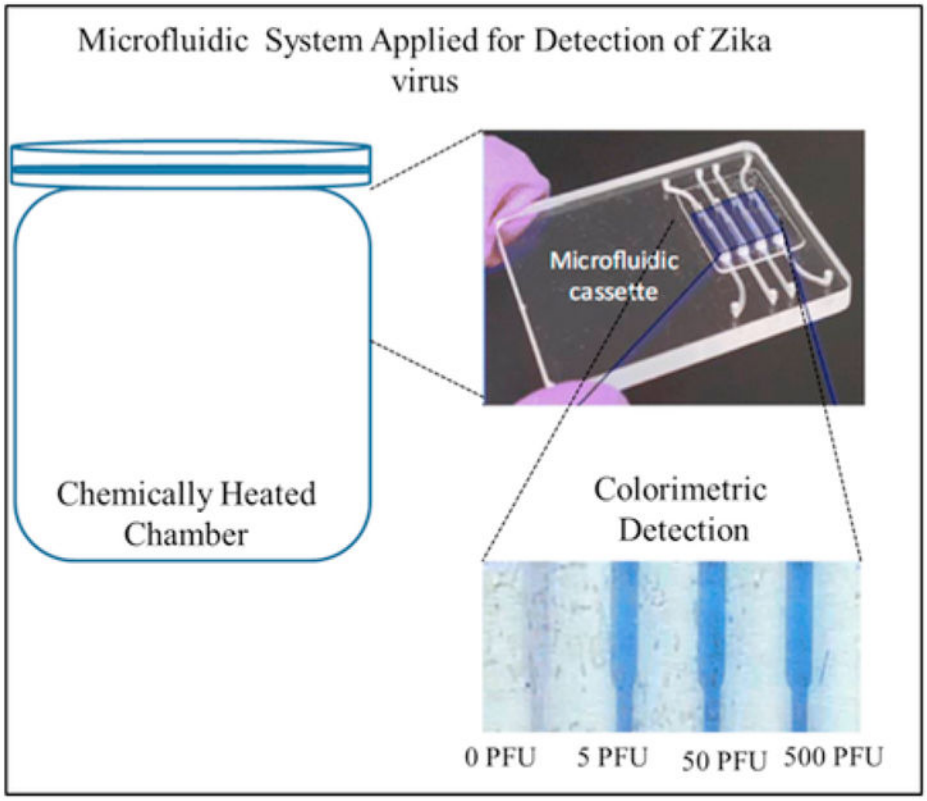

Fig. 7.

Microfluidic system based on the reverse transcription-loop-mediated isothermal amplification for the diagnosis of Zika virus (Song et al., 2016).

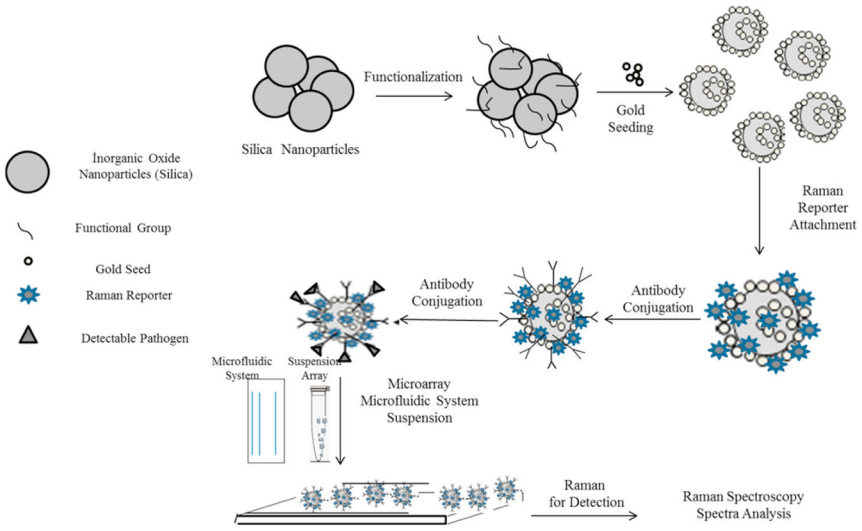

Fig. 8.

Schematic illustration of SERS based on the use of nanoparticles to detect pathogenic moieties.

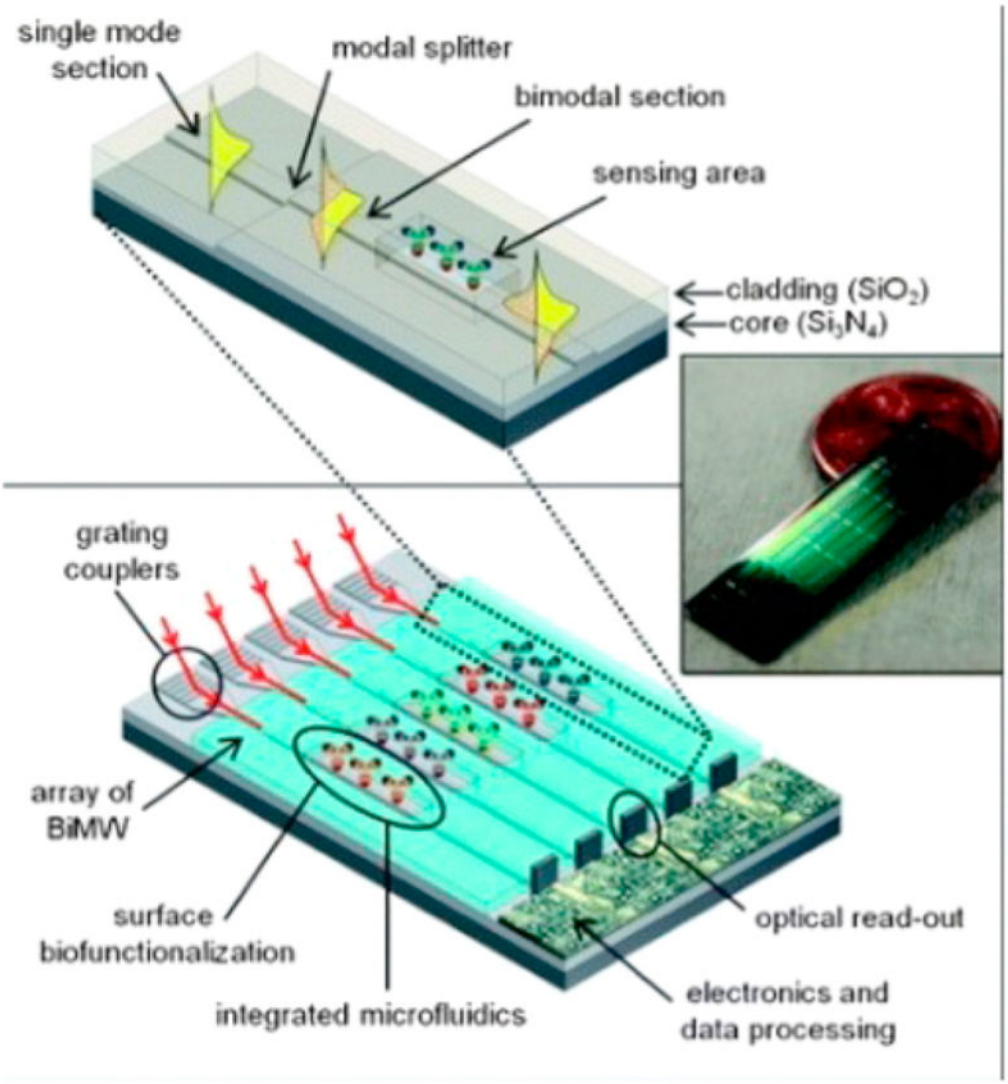

Fig. 9.

Schematic illustration of nanoparticles mediated nanophotonics LOC microfluidics system for diagnosis (Duval et al., 2012); reprinted with permission.

Table 1

Application of microfluidic systems for the detection of pathogenic microorganisms or toxins.

Name of pathogen	Research team	Detection hardware category	Targeting moiety	Sensitivity and advantage
E. coli	Moller-Kristensen et al. (2007)	Microfluidic system	Cy5 labeled antibody	Three orders magnitude improvement in *E. coli* diagnosis
E. coli	Moller-Kristensen et al. (2007)	Microelectromechanical (MEMS), SAM	DNA approach	1000 *E. coli* cells detected in 40 min
Salmonella	Moller-Kristensen et al. (2007)	Microfluidic system	Anti-Salmonella polyclonal antibodies/QDots	Sensing of 10^3 CFU/mL Salmonella cells
Vibrio cholerae	Moller-Kristensen et al. (2007)	Amplification for optical detection	–	Seven orders magnitude improvement compared to other methods
Vibrio cholerae	Moller-Kristensen et al. (2007)	RT-nucleic acid sequence-based amplification (NASBA)	Cholera toxin (ctxA), ctxA toxin regulator (toxR), hemolysin (hlyA), and the 60-kDa chaperonin product (groEL)	Applying tcpA and ctxA biomarkers for at least 3 h
Mycobacterium tuberculosis (MTB)	Moller-Kristensen et al. (2007)	Microfluidic technology coupled with nuclear magnetic resonance (NMR)	TB surface receptor	Rapid detection in microliter volumes of urine
Mycobacterium tuberculosis (MTB)	Lee et al. (2009)	Microfluidic Chip	Quantitation of rpoB gene from *M. tuberculosis*	Manipulation of small fluid volumes for detection
Mycobacterium tuberculosis (TB)	Liong et al. (2013)	Microfluidic and NMR microcoil combination	Barcoding of nucleic acid segments of TB	Rapid low-cost point-of-care detecting method in 2.5 h
HIV	Wang et al. (2012a)	Microfluidic System	DNA probe attaching to HIV	Rapid detection of HIV within 95 min (much faster than common methods)
HIV	Chin et al. (2011)	mChip design	HIV	Detection of 1 μL of unprocessed blood in case of HIV virus compared to benchtop assays
HIV	Wang et al. (2012b)	Microfluidic System	HIV-1 detection by anti-gp120 antibody	Detection of HIV-particles in 10 μL of blood under fluorescence microscope
HBV	Zhi et al. (2014)	Microfluidic Chip	Targeting of clone plasmid HBV DNA with complete genome	Detection of 10 copies/mL HBV DNA within 1 h
HBV	Zhang et al. (2010)	Microfluidic system	Targeting of HBV DNA using micro-beads and streptavidin conjugation system	High sensitivity (4 pM, S/N>3) in virus DNA detection (1000 copies/mL of HBV) in clinical serum.
Zika virus	Song et al. (2016)	Microfluidic System	Zika virus (ZIKV)	Rapid detection within 40 min by LCV dye without need for instrumentation
Zika virus	Priye et al. (2017)	Conversion to Smartphone signals	Zika virus (ZIKV)	Rapid data compilation and signal processing