

Chapter 13

Paper Microfluidic-Based Devices for Infectious Disease Diagnostics



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Abstract Infectious diseases are still a burden in the global healthcare sector. As per the WHO report, millions of people get affected annually at a worldwide level. An early diagnostic is a critical step in the prevention of their spread. The demand for point-of-care diagnostics of deadly diseases has increased significantly. From the past decades, several technologies have been developed for the diagnosis of these diseases. Among these, paper-based microfluidic devices present a unique opportunity for point-of-care diagnostic of various infectious diseases due to their simplicity, affordability, ease of fabrication, rapid detection, robustness, sensitivity and low limits of detection. As compared to laboratory-based diagnostics, the paper-based platform provides a cost-effective solution which plays a significant role in developing countries with limited resource settings. This chapter presents a brief overview of recent developments in paper microfluidic-based technology for the detection of various infectious diseases.

Keywords Infectious diseases · Paper-based device · Colorimetric detection

13.1 Introduction

In areas of scarce resources, cleanliness, and facilities, several infectious diseases are prevalent, despite the continuous struggle by the World Health Organization (WHO). Thus, timely diagnosis and monitoring of these diseases are essential for

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minimal treatment and low mortality. Modern day technological advancements in the field of public healthcare have caused a shift in the detection of viruses in clinics to kit-based detection. A need for portable, low-cost, disposable, and user-friendly medical diagnostics system has prompted the development of paper-based microfluidic devices, capable of detecting pathogenic microorganisms and chemical contaminants.

Microfluidics refers to the science of behavior, precise control, and manipulation of fluids that are geometrically constrained to a sub-millimeter scale. It is advantageous as low volumes of sample and reagent are required, and rapid detection of infectious diseases is attainable, even for the most dangerous pathogens like HIV, HBV, ZIKV, with high accuracy in identification. Paper is a reasonably intricate material, and it finds utilization in developing sensors and devices in analytical chemistry, owing to its physical properties, its availability, and easily convertible characteristics. These devices typically consist of a series of hydrophilic cellulose that steers fluids from inlet to the desired outlet. Amongst the variety of papers available, the choice of paper to use largely depends upon the fabrication steps involved in the development of a device and their areas of application. Microfluidic chips integrate preparation of samples, different reactions, separations, and identification on a microchip, which can be efficiently used as a substitute for conventional laboratories and clinical detection.

The characteristics and behavior of different microorganisms like bacteria, fungi, and viruses can be studied to examine infectious diseases caused by them and for the development of new, competent diagnostic methods, and devices. In this chapter, we focus on the dominant infectious disease-causing pathogens including *Escherichia coli* (*E. coli*) bacteria, Plasmodium parasite, Mycobacterium tuberculosis (TB) bacteria, *Vibrio cholerae* bacteria, Dengue virus (DENV), Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Zika Virus (ZIKV). We discuss the need and processes of detection of these microorganisms through microfluidics and then focus on various methods of health diagnostics. Finally, we discuss the commercialization of these microfluidic techniques.

13.2 Pathogen Detection

Detection of pathogens is one of the essential applications of paper-based microfluidic sensors in medicine since the antibiotics chosen depends upon the agent causing the disease. Here we discuss the detection of pathogens very commonly present.

13.2.1 Escherichia Coli

Foodborne diseases have been widespread across the world for decades, and they lead to extreme health, economic, and social problems. The most common

pathogen, *Escherichia Coli*, also known as *E. coli* bacteria, is mostly found in the intestines of humans and animals. The initial symptoms of being infected by these bacteria are severe abdominal cramps, fever, and vomiting, whereas, hemolytic uremic syndrome (HUS) can occur in the later stages and lead to death. It presents a challenge based on the life-threatening nature and microscopic concentration of *E. Coli* O157: H7, sufficient to cause disease. Adulterated food including ground beef, and unpasteurized milk, unclean water or close contact with the infected cause spreading of the disease. The traditional methods of detection of bacteria like counting colonies on a plate, and immunology-based methods are, although susceptible, yet time-inefficient and have complicated operation techniques. Therefore a fast, selective, and reliable technology to detect deficient concentrations of bacteria has been developed using nanomaterial-based biosensors.

Several methods of detection have been employed for qualitative and quantitative detection of *E. coli* O157: H7, such as colorimetric technique, electrochemical technique, and optical-based detection. A colorimetric biosensor uses *E. coli* proteases. The proteolytic activity these proteases is acts as a biomarker. *E. coli* O157: H7-specific peptide substrate is labeled with magnetic nanoparticles and further immobilized on a gold sensing platform. This gives a hand-held pep-tide probe in strip format. On being exposed to *E. coli* O157:H7 proteases, the magnetic nanoparticle peptide moiety is attracted to the sensor stripe, mounded with permanent magnets at the back, and as a consequence, a color change is observed (Suaifan et al. 2017). Detection by electrochemical chip provides better sensitivity and a faster response. A microfluidic chip was developed, combining dielectrophoretic and impedance analysis systems. It realized the real-time monitoring of bacteria accumulation in the cross-electrode chamber. The system automated the whole concentrating operation by remote computer control, recording the change of electrical impedance to detect *E. coli* (Zhou et al. 2019). Optical-based detection method uses a combination of fluorescence detectors and microfluidic chips. Nuchtavorn et al. introduced separation and detection using chip-based capillary electrophoresis platform with laser-induced fluorescence detection. The sensitivity of this method depends upon the capability of the fluorescent labeling reagent. A combination of aminated nanomagnetic beads and carboxyl quantum dots is used to quantify the DNA of *E. coli* O157:H7. Thus the development of fluorescent nanoprobe and quantum dots combined with micro-fluidics can cause rapid detection of this bacteria with high sensitivity (Zhou et al. 2019; Nuchtavorn et al. 2012).

13.2.2 *Plasmodium*

Plasmodium is a protozoan group which is a parasite of vertebrates and insects. These cause malaria in humans and commonly transmitted by the bite of an infected female Anopheles mosquito, called malaria vectors. Plasmodium falciparum poses the most significant threat as it causes severe illness and can lead to death. The existing tests have proven to be unreliable. It has, therefore, become a necessity to

have low-cost and rapid devices for diagnostics wherein no previous training or laboratory setup is required and can detect deficient concentrations of parasites. The difference in the deformability of healthy and infected red blood cells has been used to develop methods for malaria diagnosis. As parasites in the blood mature, the red blood cells progressively lose deformability and become stiffer. This deformability is used as a biomarker to estimate the infection stage of malaria. It is found that more stiff infected RBCs are more likely to displace to the walls of the microfluidic channels. More than 80% of the iRBCs (in trophozoites/schizont stages) could be isolated by splitting the primary microchannel into side channels. The specificity yet needs to be improved as other infections like sickle cell anemia also cause changes in the deformability of RBCs (Chen et al. 2019). In another paper-based sensors for malaria diagnostics, there are different wells containing buffer gold enhancement reagents and sample pads are kept adjacent in the design for color development by promoting lateral flow of reagents. A sample test is conducted where, on the test line, a murine antibody specific to *PfHRP-II* is coated as a capture reagent for the antigens in the sample. To visualize the binding reaction, a secondary antibody specific to *PfHRP-II* tagged with gold nanoparticles, is used as an optical tag. A gold enhancement reagent is used to improve the intensity of the signal, which bind with the GNPs. A high-intensity blue color appears, as a result of increased size of gold nanoparticles. The amplification of signals take a longer time for detection than unamplified immunoassay but the limit of detection is largely improved by this amplification, up to 2.9 ng/mL (Ragavan et al. 2018).

13.2.3 HIV

The Human Immunodeficiency Virus (HIV), belonging to the Retroviridae family is a single-stranded enveloped RNA. It is a virus that impairs the function of the immune system of the body by infecting the protective cells as it enters the body. As the infection increases, it deteriorates the immune system progressively, and the individual becomes prone to life-threatening “opportunistic infections” and cancers and leads to the Acquired Immunodeficiency Syndrome (AIDS) disease. HIV is easily transmitted through blood transfusion from the infected and re-using contaminated surgical equipment, syringes and needles, unprotected sexual intercourse, and oral sex with an infected person. During pregnancy, childbirth, or breastfeeding, HIV can also be transmitted from the mother to the infant. In the initial days of infection, no visible symptoms appear. As it progresses, symptoms like fever, diarrhea, swollen lymph nodes, and cough appear. Although the cure for HIV infection doesn't exist, yet early diagnosis and continued usage of antiretroviral drugs prevents or reduces the reproduction of the virus in the body, and thus help in maintaining health for more extended periods.

Conventional techniques used for the detection of the virus in the host are based on quantitative PCR. But these methods are expensive, require longer times and insensitive to lower concentrations of virus. Paper-based detection can be used to

determine whether the target is present in the blood or serum. The earliest biomarker after the infection is HIV p24, a capsid protein of HIV. The peroxidase-mimicking porous platinum core-shell nanocatalysts (PtNCs) is integrated into a Lateral flow immunoassay device (LIFA) to account for the sensitivity of detection. To determine the presence of the target analyte in the sample, a colorimetric detection is carried out using LFIA. In an LFIA, the core process is initiated when the sample hydrates a detection label (typically an intensely colored nanoparticle), and by capillary action, it is drawn along the paper membrane and thus have the detection label bound to the test line through a target analyte. The test line and the nanoparticles need to be decorated with antibodies (affinity ligands), which could bind different regions of the target analyte. The high sensitivity of LFIA requires faster binding reaction kinetics towards solid phase. While the sample flows past, the particle labels must bind to the test line. Through the catalytic activity of the nanoparticle, a powerful amplification mechanism is incorporated in the PtNC amplified LFIA, which increases the intensity of the signal through locally depositing dyes at the test line. In a vessel containing orthogonally biotinylated camelid antibody fragments (nanobody-biotin), and lyophilized antibody modified PtNCs, a plasma (or serum) sample is introduced. These antibody segments can bind to discrete epitope regions of the p24 capsid protein. The solution is drawn up the strip towards the streptavidin bearing test line by capillary action, using a nitrocellulose reaction membrane lateral flow strip and an absorbent pad. Higher concentrations of the target ($100\text{--}10,000\text{ pg mL}^{-1}$ p24) show a positive test result indicated by a clear black line, which occurs when particles bound at the test line are intrinsically absorbed. Catalytic amplification of the PtNCs is required for the detection of lower concentrations of the target. The oxidation of chromogenic substrate is enabled by the disproportionation of H_2O_2 , achieved by this amplification. The oxidation of substrate by PtNC generates an insoluble colored product captured on the test line. The assay can thus be easily read by eye because of the presence of the unambiguous colored product (Loynachan et al. 2018).

13.2.4 HBV

Hepatitis B Virus, of the hepadnavirus family, affects the liver in humans and causes an infectious disease known as Hepatitis B. This disease causes health problems globally, and both acute and chronic infections occur. Usually, there are visible symptoms at the initial stage. The symptoms that appear include dark urine, vomiting, and abdominal pain, nausea, and yellowing of the skin and eyes. Eventually, liver cancer and cirrhosis may develop and sometimes lead to death. Exposure to infected blood or body fluids transmits this virus. The transmission also occurs through vaginal, seminal and menstrual fluids, reuse of syringes and needles in a medical setup, and dental and surgical procedures. As recommended by the World Health Organization, vaccination for HBV is a necessity and must be done for all infants.

Simple paper-based sensors have been developed to detect HBV from blood or serum samples. Simple paper-based sensors have been designed to detect HBV from blood or serum samples. This is achieved by observing changes in the optical properties of polythiophene (PT) impregnated polyvinylidene fluoride (PVDF) membrane, when the target nucleic acid, HBV-DNA, forms a complex with PT. Orange to purple color change occurs when the complex is formed by electrostatic interactions between the nucleic acid and polythiophene (Ammanath et al. 2019).

13.2.5 ZIKA Virus

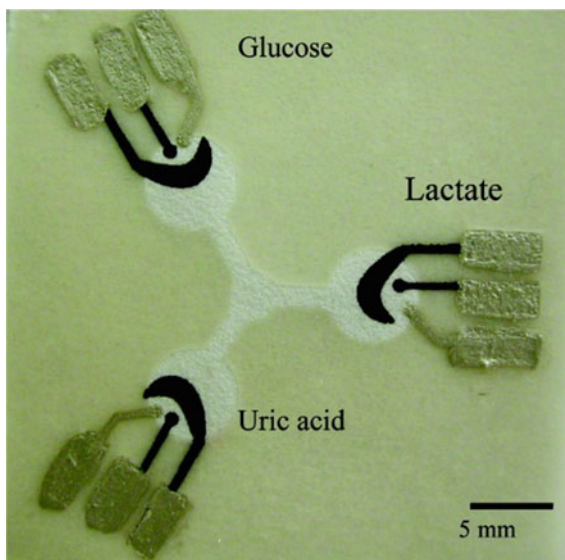
Zika Virus belongs to the family Flaviviridae, transmitted mainly by the bite of *Aedes* mosquito, which is active during the daytime. It can also be transferred to the fetus from mother during pregnancy, organ transplant, blood transfusion, and sexual contact. Zika virus was successfully isolated in the Zika Forest of Uganda, where it derives its name from. The symptoms include fever, rashes, muscle and joint pain, conjunctivitis, and headache and lasts for 2–7 days. These symptoms appear only after about 3–14 days after being exposed to the virus. Zika virus causes massive complications, especially during pregnancy, wherein it causes microcephaly and congenital anomalies in the fetus or could result in stillbirth and preterm birth. Zika virus also triggers Guillain-Barré syndrome, myelitis, and neuropathy, more commonly in adults.

A system for the rapid diagnosis of Zika infection has been developed as a response to the Zika virus outbreak. An abiotic and sterile, substrate based microfluidic system that can be used outside laboratory setup has been established, by freeze-drying cell-free transcription and translation systems onto paper with genetic sensors (Pardee et al. 2016). The RNA extracted is isothermally amplified, and freeze-dried paper sensors can be rehydrated using these. In the substrate disc, the color shift from yellow to purple indicates the detection of a suitable trigger RNA. At room temperature, these biomolecular components remain stable, and thus their storage and distribution becomes easier (Nasseri et al. 2018).

13.3 Health Diagnostics

The development in technology is carried out with the common thought; to achieve better health and living condition for the human being. Paper-based microfluidic helped a lot in gaining the lower limit of detection of different analytes with the benefits of low cost, ease of manufacturing, and flexible point of care diagnostics devices. Paper-based microfluidic devices are being constantly developed and improved for the detection of various pathogens in the water, food and biological fluids like blood, serum, urine, etc. and also for the monitoring of the glucose, uric acid and physiological parameters to monitor the good health.

Fig. 13.1 Three electrode assembly for all the three analytes. The sample is divided into three separate test zones with the help of hydrophilic channel. Reproduced from Henry et al. (2009), with permission from American Chemical Society



Henry et al. (2009) demonstrated the detection of glucose, lactate, and uric acid using the paper-based microfluidic device. They fabricated the electrochemical detection technique with microfluidics on a paper and characterization of the analytes were done with cyclic voltammetry. Screen printing technology was used to fabricate the electrodes, and photolithography was used to make the microfluidic channels on the filter paper. Glucose oxidase, lactate oxidase, and uricase enzymes are used to react with glucose, lactate, and uric acid, respectively, and the determination of analytes was done using chronoamperometry. This device shows the integration of electrochemical detection with the paper microfluidic as a robust alternative for the point of care monitoring. Figure 13.1 shows the paper-based device fabricated by Henry et al. consisting of hydrophilic channels and three separate testing zones.

Takahashi and team (Takahashi et al. 2018) fabricated a synthetic platform for the analysis of microbiome sample. They address the need for a simple and affordable paper-based platform to analyze microbiome sample which is responsible for many biological functions of the body. mRNA from 10 different bacteria which affect human health and four clinically relevant host biomarkers were detected using RNA toe-hold switch sensors and compared with the RT-qPCR. Further detection of toxin mRNA in the diagnosis of *Clostridium difficile* infections was highlighted. Yang et al. (2018) worked for the health and wellbeing of cattle by detecting sexually transmitted infection (STI) pathogens using paper-origami DNA microfluidics. They tested the semen samples from elite bulls and detected three bovine infectious reproductive diseases through fluorescently. The paper device was fabricated by hot wax print-ing five channels on the paper, which includes positive and negative controls and three pathogen targets. There were three

components in each device; wax printed filter paper, single-sided adhesive acetate film sealed plastic plate with five paper spots and glass fiber disk to adsorb nucleic acid from the sample in the case of high concentration of the chaotropic agent, guanidine thiocyanate. Pathogen DNA from one viral pathogen and two bacteria (bovine herpes virus-1, Brucella, and leptospira) was extracted, amplified, and detected. This data was either collected visually or digitally with a mobile phone camera. The limit of detection with this device was also evaluated using pathogen-inoculated semen sample and found to be as low as 50 leptospira organisms, 50 CFU Brucella, and 1 TCID50 BoHV-1. Figure 13.2 represents different parts of the device and five separate reaction chambers, one each for the detection of three analytes, positive and negative control.

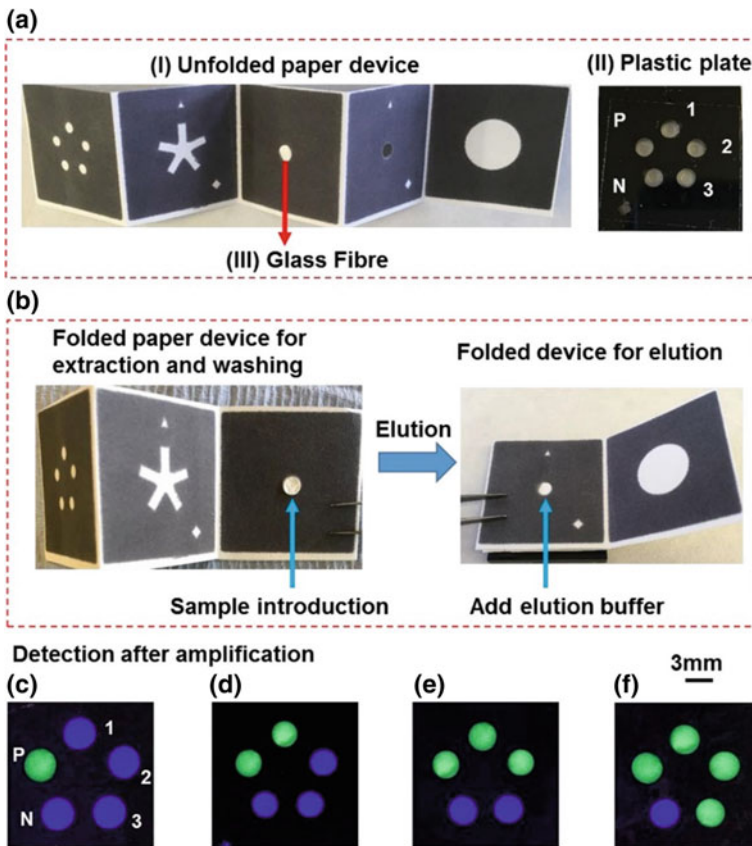


Fig. 13.2 Design of the paper device having five different zones for positive, negative control and for detection of three targets. **a** Shows the unfolded filter paper, plastic plate having five reaction chambers and glass fiber. **b** Sample introduction into glass fiber followed by washing and elution (**c–f**) detection of different analytes. Reproduced from Yang et al. (2018), with permission from American Chemical Society

Kumar et al. (2018) reported a novel lateral flow immunoassay for the detection of dengue NS1. They used the tapered flow design for paper microfluidics simultaneously leveraging on the benefits of Au-rGO as the detection label. rGO used provides a high surface to volume ratio inhibits the gold nanoparticle aggregation, which eventually increases the number of active binding site for the anti-dengue antibodies. Due to this modification, the concentration gradient of the antigen bound nanoparticles at the test line increases, which increases the color intensity. This enhancement of the signal enables them to reach the extremely low detection limit of 4.9 ng/ml. The taper angle of the device has been optimized to achieve the high sensitive limits and rapid detection (10 min) of NS1. The method has been demonstrated with spiked in human serum sample and resulted in the 11 fold improvement over the previously reported values. This lateral flow immunoassay can be used for the rapid detection of several target diseases at an early stage.

Suaifan et al. (2017) developed a novel biosensor for the detection of *Escherichia coli* O157:H7 through a peptide probe. This device is based on the ability of *E. coli* proteases to change in the optical response. The gradual increase in the golden color, which is visually observed is due to the surface modified, magnetic nanoparticles. The color intensity and concentration of the *E. coli*. are correlated and is quantified using image analysis software. The detection is with magnetic nanoparticle-specific (MNP-specific) peptide probe, with a limit of detection as low as 12 CFU/ml in both samples and 30–300 CFU/ml in a spiked food sample. This sensor is rapid, easy to use, low cost and easy to mass produce and thus, can be used with the regulatory authorities and by the food manufacturer for better control of the food quality and health. Figure 13.3 represents the schematic of the fabrication technique of the colorimetric biosensor to detect *E. coli* O157:H7.

Similar such attempt has been reported by Bo Pang and team where they developed a paper based ELISA test for the detection of *E. coli* O157:H7. This enzyme-linked immunosorbent assay (ELISA) device operates with shorter operation time, higher sensitivity, lower cost and lower limit of detection (10,000 CFU/ml). The filter paper was pretreated with chitosan and glutaraldehyde to increase the bacteria immobilization and then incubated with anti-*E. coli* primary antibody. Horse radish peroxidase conjugated anti-goat secondary antibodies are also incorporated in the paper. With the tetramethylbenzidine-hydrogen peroxide (TMB-H₂O₂) solution, the color signal was obtained and is analyzed by imageJ software (Pang et al. 2018). Figure 13.4 illustrate various steps involved in the fabrication of p-ELISA based biosensor for the detection of *E. coli*.

Rengaraj et al. (2018) developed a rapid, low cost, on site screening method for the pathogenic microorganism in the drinking water. They fabricated carbon electrodes onto hydrophobic paper and used carboxyl group to functionalize electrodes. Concanavaln A (Con A) is used as the biorecognition element and device is tested as impedimetric sensor for bacteria in water. This device can detect bacteria up to lower level as 1900 CFU/ml. This portable paper based device shows linear

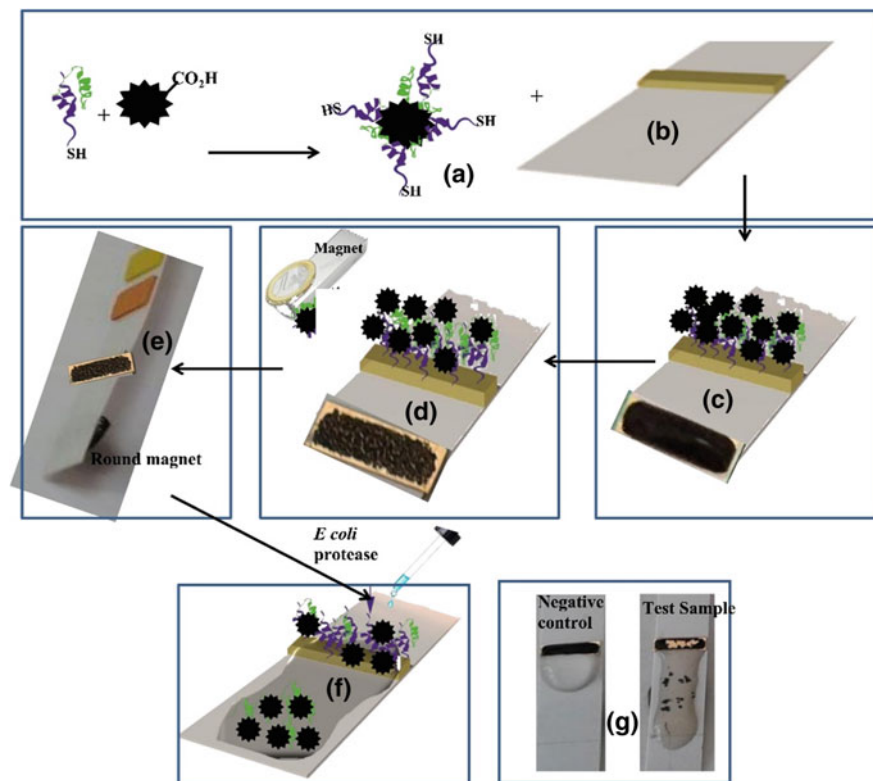


Fig. 13.3 Schematic representation on the actual image of the colorimetric biosensor to detect *E. coli* O157:H7. **a** *E. coli* O157:H7 specific peptide substrate functionalized with magnetic-nanoparticles (MNPs). **b** Gold sensing platform. **c** Immobilization of the sensing monolayer. **d** Removal of unattached MNPs by external magnet. **e** Side view showing magnet attachment. **f** Sample addition. **g** Detection of *E. coli* and negative blank control on paper based biosensor. Reproduced from Suaifan et al. (2017), with permission from Elsevier

increase in the probe charge transfer resistance with bacterial concentration ranging from 10^3 to 10^6 CFU/ml. Sun and team (2018) fabricated a paper based analytical device with combining PVC pas and filter paper for the detection of cronobacter spp. Pathogen in food and water. This device consist of a micro spot which changes color on the bacteria detection from colorless to indigo due to species-specific enzyme association. The testing time of this device is 10 h or less and is capable of detecting live bacteria with concentration as low as 10 CFU/cm².

For the early diagnosis of plasmodium falciparum malaria, Santos et al. developed a paper based platform with minimal amount of reagents for detection of histidine-rich protein. The device consist of different regions for sample addition, detection zone and absorbent pad, all connected with microfluidic channels for

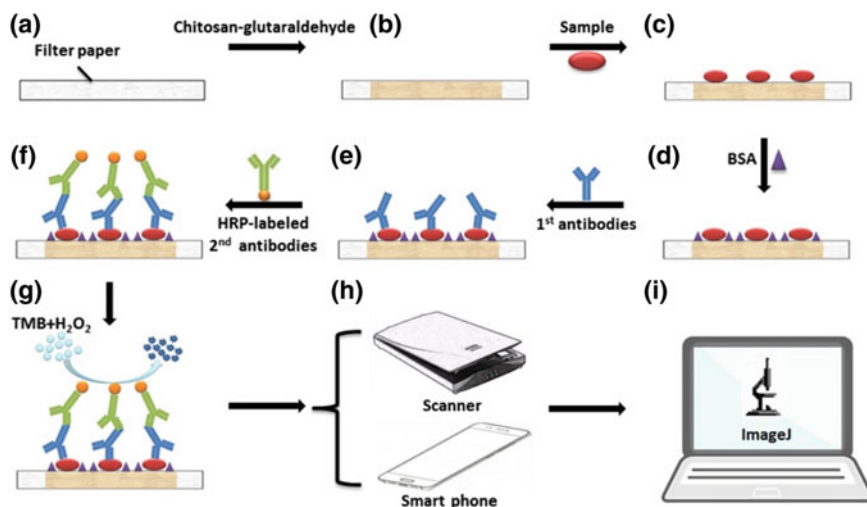


Fig. 13.4 Schematic representation of paper-ELISA. **a** Selection of Whatman #1 filter paper; **b** pretreatment of paper with chitosan-glutaraldehyde; **c** attaching the sample on the paper; **d** blocking with the help of BSA; **e** Application of primary antibody; **f** incubation of secondary antibody labelled with HRP; **g** color development with TMB; **h** data collection as an image using scanner or printer; **i** analyzing acquired image. Reproduced from Pang et al. (2018), with permission from Elsevier

performing lateral flow assay. This whole assembly is fabricated through CO₂ laser cutter. This modified shape causes delay in flow at the detection zone as compared to the conventional lateral flow systems which provides adequate time for reaction. With the captured antibody reactions, this device shows good sensitivity and detectability visual and calculated limit to be 5.0 and 4.5 ng/ml that is sufficient to identify the disease at early stage and thus, cutting down the requirement of nanostructures or other amplification techniques (dos Santos et al. 2018). Figure 13.5 represents the overall layout and schematic for the fabrication of paper based device for detection of malaria.

Colleen N. Loynachan and team developed a paper based lateral flow immunoassay for the detection of p24, one of the earliest and most conserved biomarker of HIV. This novel device is serum stable and consist of porous platinum core-shell nanocatalysts (PtNCs) which surpasses the current commercial and published sensitivities of paper based detections. The specific antibody has been modified with platinum nanocatalyst to have high specificity and affinity towards p24. This catalytic activity enable the naked eye detection of p24 biomarker in the spiked serum sample even in the low femtomolar range and reduced the detection time to less than 20 min (Loynachan et al. 2018).

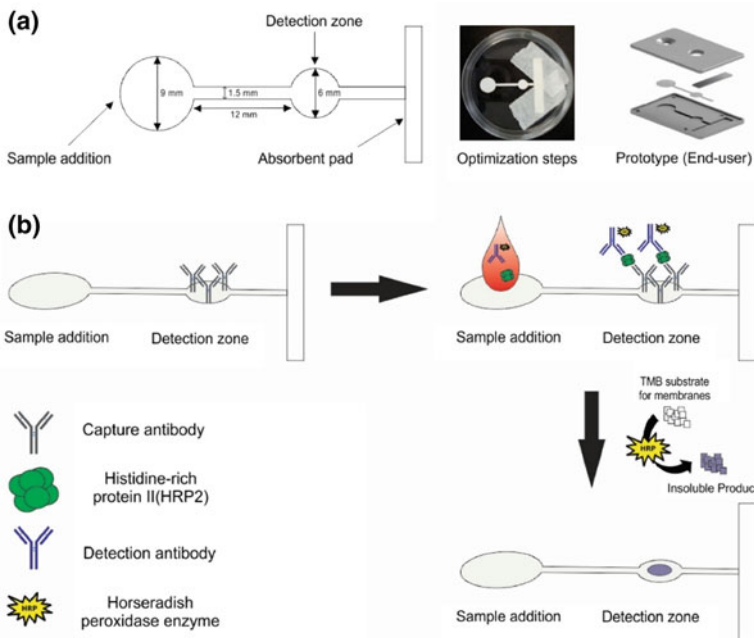


Fig. 13.5 **a** Overall layout of the device; designing, optimization and prototyping for end user. **b** Schematic for the preparation of paper based platform for diagnosis of *Plasmodium falciparum* malaria. Reproduced from Sun et al. (2018), with permission from Elsevier

13.4 Commercialization and Challenges

With the development of newer technologies for the point of care diagnosis, commercialization plays a very critical role in providing access to this technology to in need community. Most of these commercial technologies available are based on either antibody or DNA primer although various other means are available for diagnostics purpose. The devices having these analytes are much easier to modify to cater to different variety of diseases and thus covering a broader spectrum of diseases (Su et al. 2015). Table 13.1 shows the commercially available microfluidic-based system for point of care infectious diseases diagnostics.

However, implementation of this ideation on a commercialize stage is full of various challenges. These innovative platforms are designed with the thought to cater to the resource scare part of the world, and thus, pricing of the device should be low. Due to this low pricing, the profit associated with the product is also small, which is not appealing to many profit-seeking venture capitalists. This is one of the biggest hurdles in the way of commercialization. Luring other financial support from the non-government organization and private, not-for-profit organization working in the health domain becomes the only option for many groups.

Table 13.1 Commercially available microfluidic-based system for POC infectious disease diagnostics

Pathogens ^a	Analytes	Technical features	Company ^b	Website
Bacterial and viral	Primer	Disposable cards with benchtop analyzer, on-card sample processing	Cepheid	cepheid.com
Influenza, Bacterial	Primer	Portable detector (3 M™ Integrated Cycler), discs with on-board extraction	Focus Dx (Quest)	focusdx.com
Bacterial	Primer	Disposable cards with integrated heating, detection, sample processing in a portable instrument	HandyLab (BD)	bd-world.com
Viral	Primer	Automated analysis on FilmArray™ instrument	Idaho Technologies	BioFireDx.com
Bacterial, HIV/AIDS	Primer	Lab-in-a-tube platform for automated analysis	IQuum	iquum.com
Bacterial	Primer	Compact tabletop analyzer utilizing membrane-based quantum dot detection	LabNow	labnow.com
HIV/AIDS, MRSA	Primer or Antibody	Compact, benchtop analyzer, manipulation of nano- and micro-droplets	Advanced Liquid Logic	liquid-logic.com
Viral	Primer or Antibody	Disposable plastic discs (spinit®); label-free, portable SPR analyzer	Biosurfit	biosurfit.com
HIV/AIDS, hepatitis, flu	Primer or Antibody	Integrated fluidic cartridge and low-cost, low-power fluorescence imaging using planar waveguides	Mbio Diagnostics	mbiodx.com
Malaria	Primer or Antibody	Disposable cartridges composed of thin-film laminates and injection-molding	Micronics (Sony)	micronics.net
HIV/AIDS	Antibody	Disposable cartridge, portable analyzer, automated image based immune hematology test	Alere	alere.com
Bacterial, HIV/AIDS	Antibody	Portable analyzer, injection-molded plastic cassette, low cost optical detection of silver films	Claros Diagnostics	opko.com
Bacterial, HIV/AIDS	Antibody	Compact tabletop fluoro cytometry instruments with disposable cards	LeukoDx	leukodx.com

^aMRSA, Methicillin-Resistant *S. aureus*; HIV/AIDS, Human immunodeficiency virus infection

^bAcquisitions of POC microfluidics companies. Reproduced from Su et al. (2015), with permission from Elsevier

Government and philanthropic foundation can play a very crucial role in research, development and finally in rolling out the product in the market (Tay et al. 2016).

Apart from finances, another major issue for the commercialization run is the integration of different technologies on a single platform. Commercialization for point of care diagnostics needs a robust product which is easy to use by the patient itself. This “easy to use” is different from working in the lab and working on the field. In the lab, there are many resources available which might not be readily available on the field and thus, make the use of the product little uneasy. This is one of the major problems which is not catered by many research groups working in this field and formalizing a startup for diagnostics. Also, various technologies like fluid delivery, on-chip storage of sample and results, heating/cooling, pre-treatment of sample like dilution, mixing, etc. has to be combined with the detection technology to fabricate a fully functional product (Chin et al. 2007).

Other challenges that become very important for commercialization are approval from the regulatory authorities. The criteria for these approvals are different in different countries which are based on the socioeconomic status of the country, market availability for the product, and existing competition for the new product. Although some criteria that are generally followed by various authorities for point of care diagnostics applications are fully automated test, use of unprocessed specimen, no specialized training required to operate, results easy to interpret and unaffected by environmental conditions. Apart from fulfilling these criteria, the pathway of commercialization in many developing countries is very slow due to which the newer technology becomes old when it gets into the commercialize stage. Although this problem can be tackled by coordinating between different NGOs, government agencies and authorities (Chin et al. 2012). Figure 13.6 represents a technology map of design choices made by selected microfluidic companies.

In many countries, government bodies are taking many initiatives to help various startups and small companies to get them closely connected to government functioning and also to help them gel well among themselves and with different manufacturers, raw material suppliers, etc. These schemes help the government to monitor all the groups associated with the commercialization of point of care diagnostics to provide help or intervene whenever required.

13.5 Conclusion and Future Perspectives

The WHO is aiming at the complete elimination of infectious diseases through affordable and sensitive diagnostics. The limitations of existing conventional methods used for infection diagnosis, such as higher costs, technical complexities, longer processing time and poor sensitivities can be overcome by “smart” paper-based sensors (Tay et al. 2016; Liana et al. 2012). Paper-based microfluidic techniques can be successfully implemented as an economical and reliable manner. In developing countries, the number of trained personnel and equipment are deficient, and hence, the development of paper-based portable devices can potentially

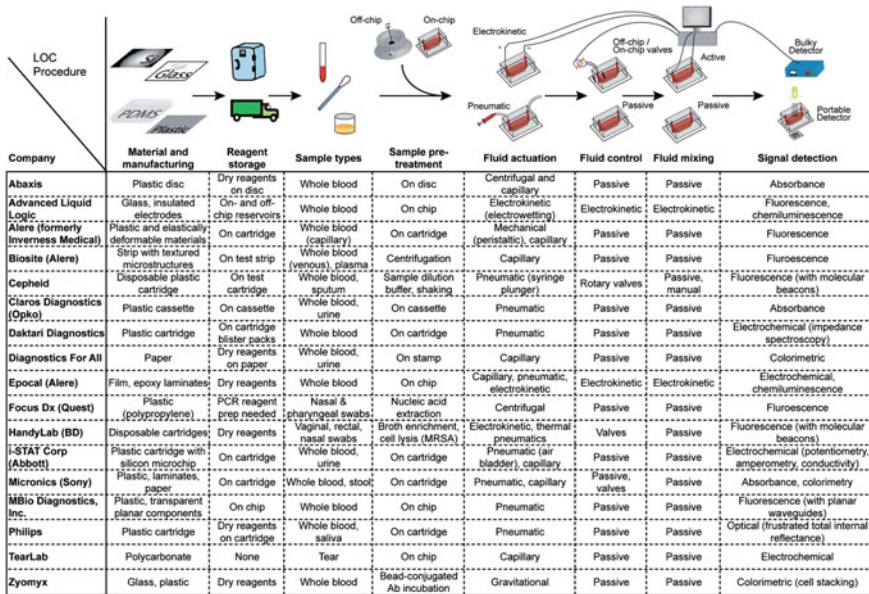


Fig. 13.6 Technology map of design choices made for a set of common procedures central for production of integrated point of care diagnostics device by selected microfluidics companies. Reproduced from Chin et al. (2012) and Chin et al. (2007), with permission from Royal Society of Chemistry

help patients. These devices use high-tech biomarkers, and bioassays, which are cheap and thousands of reactions required for linking reagents to specific microorganism is implemented in minutes. Extensive research work shows that the detection of various disease-causing pathogens can be done with great precision and accuracy using only small volumes of sample and high sensitivity and integrability can be achieved with minimum limits of detection. However, these can be improved to achieve direct detection of complex diseases and viruses and their quantitative estimation (Lim et al. 2017). It is also required to remove the pretreatment steps for several detections to increase the rapidity of the test. Related clinical symptoms are present for several infectious diseases; a device with multiplex functionality is also desirable. For a specific contagious disease, characterization of a set of biomarker signatures may be a significant advancement in future biomarker screening (Chen et al. 2019). With advancing techniques, new areas of application come into the picture. It is expected that microfluidic detection and analysis will help in optimization of drug doses and help personalize medicine for more than one disease. The challenges, including fabrication techniques and incorporating different materials and markers for different detection techniques onto paper, further research needs to be done. Multidisciplinary collaboration, including industry sciences, researchers, managers, and clinicians can accelerate developments in this field.

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