

Chapter 14

Low-cost Paper Analytical Devices for Environmental and Biomedical Sensing Applications

H. Manisha, P. D. Priya Shwetha and K. S. Prasad

Abstract Over the last decade, the fabrication of analytical devices utilizing microfluidic structures and lab-on-a-chip platforms has shown breakthrough advancements, both for environmental and biological applications. The ASSURED criteria (affordable, sensitive, specific, user-friendly, robust, equipment-free, delivered), developed by the WHO for diagnostics devices, point towards the need of paper-based analytical devices (PAD) for diagnostics. On the other hand, cost-effective PADs owing the great advantage of affordable applicability in both resource-rich and -limited settings are recently employed for on-site environmental monitoring. In this book chapter, we will discuss about the brief history of paper analytical devices, fabrications, need, and its environmental and biomedical applications.

Keywords Paper analytical device · Point-of-care · Biomarkers
Pesticides · Organic pollutants · Sensing

1 Paper Analytical Devices

Microfluidic devices provide innovative solutions to logistical problems, affording the advantages of high sensitivity, low cost, low reagent usage, small size, and several established fabrication techniques (Sackmann et al. 2014). Plethora of these devices has been used as a lab-on-chip type sensor for many biologically important molecules (Cate et al. 2014; Tomazelli Coltro et al. 2014). Among the developed microfluidic devices, PADs have tremendous amount of research attention due to their simplicity, capillary-based pumping ability, and low cost and easiness in

H. Manisha and P. D. Priya Shwetha: Equal contribution by these two authors.

H. Manisha · P. D. Priya Shwetha · K. S. Prasad (✉)
Nanomaterials Research Laboratory (NMRL), Nano Division—Yenepoya Research Centre,
Yenepoya University, Deralakatte, Mangalore 575018, India
e-mail: ksprasadnair@yenepoya.edu.in

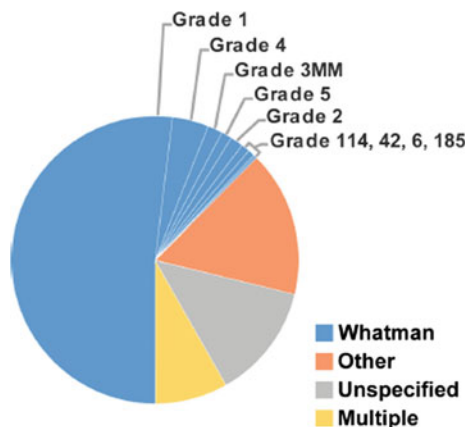
availability to resource-limited settings (Yetisen et al. 2013; Pelton 2009; Hu et al. 2014; Martinez et al. 2009). On the other hand, the capability of filtering the samples to remove the particles and aggregates, helpful surface chemistry for easy modification, biocompatible nature and ability for a safe disposal by incineration makes the PAD as the holy grail in POC diagnostic devices (Cate et al. 2014; Pelton 2009). The PADs are usually incorporated with suitable transduction methods like optical and electrochemical techniques for quantitative analysis. Mostly, the PADs for diagnostics purpose utilize colorimetric, electrochemical, chemiluminescence, electrochemiluminescence, electrical conductivity, and surface enhanced Raman spectroscopy techniques (Cate et al. 2014; Yetisen et al. 2013). Among these, visual identification of the results through naked-eye colorimetric determination is promising, while considering the resource-limited set-up as it does not require a sophisticated instrument or a skilled person. Perhaps the requirement of a higher analyte concentration, and only the yes or no results, makes the colorimetric system unsuitable for ultrasensitive detection. To overcome such limitation and bottlenecks, much sensitive electrochemical detection and signal amplifications strategies are useful (Taton et al. 2000). Müller and Clegg (1949) used an embossing tool to form patterns of hydrophobic and hydrophilic domains on a piece of paper in 1949 to develop two-dimensional PADs. Nevertheless, paper-based devices got much attention after the advent of George Whiteside's group at Harvard, on construction of microfluidic paper-based device for chemical analysis in 2007 (Martinez et al. 2007). A patterned paper platform by employing photolithography technique has been used to develop colorimetric sensor for glucose and protein in urine. After the introduction, there has been a surge in the paper-based diagnostics research. Paper-based indirect ELISA also has been developed (Cheng et al. 2010).

2 Fabrication of Paper Analytical Device

For developing paper-based devices, various types of papers are used and they can be nitrocellulose, cellulose paper, etc. The selection of the paper for fabricating the devices depends upon the procedure and the field of application of the particular fabricated platform (Fig. 1).

Even though different grades of paper exist, very few have been used for the fabrication of microfluidic PADs. Whatman brand filter and chromatography papers, in particular, have developed “gold standard” status in the field. A recent evaluation by Charls Mace group had noted that Whatman grade 1 has been used in most of the PADs. However, it should be noted that interestingly some of them did not identify the exact type of the paper used for PADs (Fernandes et al. 2017). In various fields of application, cellulose paper has been used by the researchers since it is easily available, capillary movement of the liquids through it, and the hydrophilic properties (Apilux et al. 2010). Since the paper substrates are highly porous in nature, electrodes can also be developed by screen printing and can be used for the development of electrochemical sensors (Nie et al. 2010). Nitrocellulose paper,

Fig. 1 Types and grades of paper used to fabricate paper analytical device (Fernandes et al. 2017)



which is hydrophobic in nature, can also be used as a paper substrate for the fabrication of PADs. It has a smooth surface and with a uniform pore sizes ($0.45\ \mu\text{m}$) which makes it as a good substrate for stable liquid flow. Also these papers have the provision for immobilizing DNA, since they have huge non-specific binding affinity towards biological entities (Cretich et al. 2010). In addition, paper has been used as a substrate because of its white background which allows the colour changes to be detected by naked eyes, and also it allows the uniform distribution of the samples through the capillary action. In addition, after patterning paper could be used as a substrate for sample development, purification, product formation, and other reactions may also take place without any contamination issues. Different designing approaches are used for fabricating paper devices. Generally paper is treated with polymers or SU8 for developing designs or patterns, and stacking of reagents on the surfaces of paper substrate can be achieved by inkjet etching or printing, wax printing, screen printing, and flexography printing. Among others, the principle approaches used for incorporating chemical alteration of the paper surface is by plasma treatment and inkjet printing (Dou et al. 2015). All these designing approaches have their own merits and demerits, and the selection depends on the application. Figure 2 depicts the various fabrication methods involved with PADs by using handcrafted devices fabricated using (A) wax drawing, (B) polymer ink drawing or stamping, or (C) wax stamping. Masks were used to protect hydrophilic regions for (D) wax dipping, (E) photolithography, and (F) wax screen-printing. Fabrication techniques with ink addition printers used either (G) wax printing, (H) inkjet etching, (I) inkjet printing, or (J) flexographic printing. Cutting or shaping air boundaries or etching channels were performed by a (K) craft cutter or (L) laser cutter (Cate et al. 2014; Martinez et al. 2008).

Blocking of pores in paper substrates can be done by physical approaches, and photolithography technique has been used for designing a paper-based microfluidic gadget, which requires a copier machine or an inkjet printer, UV light, and a hotplate. Also, time taken to complete this action is less than 30 min (Martinez et al. 2008).

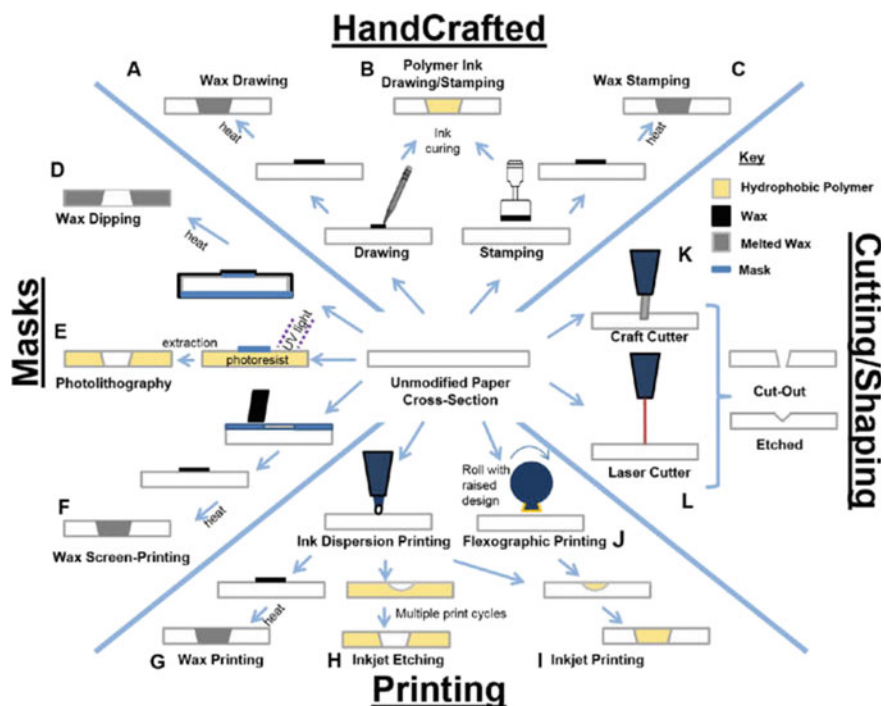


Fig. 2 Different ways of hydrophobic barrier fabrications (Cate et al. 2014)

Photolithography relies on UV exposure through a photomask of photoresist (SU-8 or PDMS)-saturated paper. The photolithography procedure requires natural and organic solvents during developing, and during the usage of these solvents there may be chances of harming the inherent properties of the paper substrate, hence making the manufactured paper devices less flexible and may end up in collapsing or bending. So when compared with the multistep photolithographic method, plotting or printing is a way to characterize hydrophobic designs on paper by printing the hydrophobic polymer. PDMS is ordinarily utilized as a part of the plotting procedure since it is affordable and less toxic. The use of PDMS was first examined by Bruzewicz et al. (2008) and used a plotter to print a hexane solution of PDMS on the filter paper as hydrophobic hindrances. The above said particular approach will not harm inherent properties or flexibility of the paper, but a time consuming approach will not allow a controllable penetration of PDMS. Nevertheless, cost of photoresist and the potential for background reactivity makes photolithography fabrication method less desirable.

Another method to create barriers in paper itself is wax printing method. When compared with the multistep photolithography technique, wax printing has many advantages like they are less complex, more affordable, and more adaptable for various changes. Whitesides' group proposed wax as another hydrophobic material

for fluidic obstructions or blocking the pores on the paper (Carrilho et al. 2009). It is also cost effective, easily available, non-harmful, easily degradable, water insoluble, and also flexible at ambient temperature. The procedure is also quick (5 min) and does not require any solvents. Parafilm was one of the earliest materials used to pattern paper for simple colorimetric metal spot tests using a heated metal stamp (Yagoda 1937). Though wax is convenient to use, it has some limitations, like the softened wax spreads easily in every direction by capillarity, which make the designs poorly characterized and also off colour in high resolution. But the spreading distance is consistent for a given heating time at a specific temperature (Lu et al. 2009). The use of vacuum could limit parallel spreading of the wax through the paper substrate. It should be noted that wax is water insoluble yet dissolvable in non-polar organic solvents; thus, PADs could disintegrate in organic solvents if it is fabricated by wax printing. Alternative barrier materials can provide a wider range of chemical compatibility. Jahanshahi-Anbuhi et al. (2015) used pullulan, a polysaccharide polymer, as a barrier to organic solvents in tandem with wax barriers to fabricate omniphobic devices. Variety of hydrophobic materials have been used in designing microfluidic PADs, with fluoropolymer (Chen et al. 2013) and octadecyltrichlorosilane (He et al. 2013). Barriers made from the aforementioned materials contain water and variety of organic solvents, so the worry of disintegrate in organic solvents is negligible. Microfluidic PADs developed using hydrophobic methylsilsequioxane showed resistance to pH-induced hydrolysis and also was found to be compatible with cell lysis solutions used, which has been reported by Wang et al. (2014).

Screen printing is simpler and sufficient method for developing nations when compared with wax printing, due to the high cost occupied with wax printers and the accessibility. Screen printing system needs a mesh. Strong or solid wax can be traded within the mesh while it is squeezed. The wax used for printing purpose was dissolved and moulded into the paper surface to form hydrophobic hindrances or the barriers with the help of a hot plate. Wax screen-printing is a minimal cost, straightforward, and quick technique for creating paper-based microfluidic gadgets which is published by Dumgchai et al. (2011). In addition, with simplicity in mind, a single-method screen-printing strategy for designing hydrophobic polystyrene could be used to create barriers. The solution of polystyrene is applied on the screen, infiltrates through the paper, and hence forms a 3D hydrophobic hindrance or barrier, forming a hydrophilic detection zone (Sameenoi et al. 2014).

Flexographic printing is also another method that designs or patterns polystyrene barriers onto chromatographic paper, enabling roll-to-roll channel patterning. Polystyrene is important among the most prevalent hydrophobic agent utilized as a part of this system because of its biocompatibility and the advantage of having not the requirement of heat treatment. Polystyrene patterns are shaped on the surface of paper with polystyrene ink of strength 5%, which incompletely enters the paper of interest. On the rear end of the paper, uniform layer of polystyrene is applied, which serves as a waterproof layer to the whole paper used for fabrication purpose (Olkkonen et al. 2010).

Selective removal or modification of the hydrophobic material after deposition has also been investigated in processes such as inkjet etching and plasma treatment.

To turn the exposed area of hydrophobized paper made with alkyl/alkenyl ketene dimer to hydrophilic, plasma treatment has been used (Li et al. 2008, 2010). Salentijn et al. (2016) showed this as well as subsequent regeneration of hydrophilic behaviour in the paper after oxygen plasma treatment.

Other techniques like laser printing and paper cutting are the techniques used for developing hydrophobic regions on the paper substrates. Easier strategy to manufacture PADs is paper cutting and taping. Cutting approach is fast, requires only scissors, and may be also PC-based gear or laser cutter. Cutting is reasonable for shape formation during manufacturing and is also perfect with cellulosic substrates (Martinez et al. 2008). Craft cutter has been developed and used to form paper boundaries, then by overlaying the paper with films of thermoplastic by Fan group (Liu et al. 2013). Cutting/lamination approach has been used to develop laminated paper-based analytical devices (LPADs) and is fundamentally the same as developing any card proofs and also has important added advantages like non-tedious and also rough help. Transporter or carrier sheet is used as a conciliatory layer and also enables slicing to be done in a solitary stride or in a one-step approach without damaging the paper used for fabrication. This method does not make use of costly laser cutting equipment and also is widely adaptable compared to special craft punch. In the case of laser-based patterning, the hydrophobic barriers are established by the polymerization of the photopolymer. In addition, it is possible to cut a strip of paper using laser as indicated by pre-designed patterns. Laser direct writing (LDW) techniques have also been shown to form hydrophobic barriers by polymerizing photopolymer-impregnated filter paper, and this approach has been applied to fabricate time delay barriers. It is also possible to create delay barriers in filter paper by adjusting the write speed of a laser to control the barrier height, which resulted in the delayed flows of different solutions throughout the device (He et al. 2015). Creating or developing economical microfluidic designs on paper utilizing laser method was reported by Chitnis et al. Those papers developed using hydrophobic surface covering layers (e.g. wax, parchment, and palette) are utilized for creating cost-effective microfluidic patterns. The structure and the properties (hydrophobic to hydrophilic) of these papers used for fabricating devices were modified selectively by utilizing a laser which is of CO₂ (Chitnis et al. 2011). Owing to its advantages, the laser patterning is not so feasible considering the cost associated with it.

Stamps, to impress a particular pattern using hydrophobic ink, also are used to make hydrophobic barriers onto paper. However, it should be noted that stamping method could jeopardize the quality of PAD, since the possibility of spreading of hydrophilic ink (Carrilho et al. 2009). Stamp with geometry of hydrophobic PDMS barrier along with images of side-by-side comparison showing advantage of PDMS barriers with respect to wax is reported by Dornelas et al. (2015). By heating and applying pressure to transfer hydrophobic materials onto PAD could be done by employing embossing technique. Different hydrophobic materials could be used for embossing along with “ink paper”, otherwise by employing laser-induced forward transfer (Jiang and Fan 2016).

3 PADs for Environmental Analysis

Cheap and best, quick, versatile, selective, and specific gadgets are important for on-site environmental checking. For developing such gadgets, PADs are considered as suitable due to the following aspects: (a) it is amazingly cheap and commercially accessible; (b) fluids can be transported by capillary strengths, and along these lines it does not require outside force or an external pump; (c) it is easy to be designed; and (d) it comprises of permeable cellulosic fibre systems that make it bio-friendly. Biological elements (proteins, antibodies, bacteriophages, and aptamers) are the well-known recognition components widely used for the development of PAD-based biosensors, since it shows high selectivity towards target sample of interest. In this manner development of bioactive PADs found great degree of use as efficient and modest sensors.

Despite, most of the developments in PADs are focused more onto health care related diagnostics and are focused to reduce the cost, and to have more benefits to common man. Nowadays other fields such as environmental monitoring, explosives detection, and screening for food and beverage contamination are also found applications of PADs. As of late, more efforts have been put to utilize PADs for environmental monitoring. Accurate and low-cost monitoring is important for environmental applications, where routine testing is conducted for the analysis of river, soil and air contaminants. Herein, we are giving a glimpse of the paper analytical sensors reported for the environmental monitoring of metals and non-metals, organic molecules, pesticides, and microorganisms.

3.1 *Metals and Non-metals*

Environmental metal contamination is common in three sources, air (aerosols), water, and soil, and many paper analytical devices have been developed for metal detection because of their known toxicity. Metals such as Fe, Cu, Cr, and Co are redox active and have capacity for creating free radicals, which further produce oxidative stress in life forms. On the other hand, Pb and Cd are known for their neurotoxicity (Meredith et al. 2016). Exposure to hazard metals causes numerous health issues; however, progressing endeavours to recognize sources of exposure is impeded due to high cost of estimation, which bringing about restricted tests or monitoring options. Since 2010, PADs for metal detection have got great consideration due to the fact that coloured metal–ligand complexes are effectively able to recognize by naked eyes and measured cheaply using other optical patterns. Microfluidic PAD-based measurement of metals such as Fe, Cu, and Ni in n air-borne particulate matter was reported by Mentele et al. (2012) with a detection limits of 1–1.5 μg for each analyte. In addition, the same device has been used to detect Cr and Cr (VI) from ash and welding fumes (Rattanarat et al. 2013). Despite the development in colorimetric detection methods, the detection limits of having

“ppm” levels make these methods undesirable, where a “ppb” level of detection is required for much better sensitive assay. Interestingly paper gadgets by colorimetric means have been fabricated for measuring other toxic metals such as Zn, Cu, Ag, Cd, Pb, Ni, Hg, and Cr (VI) utilizing metal-to-ligand charge-exchange chemistry (Meredith et al. 2016). In order to circumvent the bottleneck of having less sensitivity and ppm levels of detection, electrochemical assay methods are used and it has the ability to quantify metals at sub-ppb levels. So a combination of colorimetric and electrochemical detection is found to be an answer for the less sensitive colorimetric methods (Apilux et al. 2010). A paper sensor by combining both electrochemical and colorimetric detection for the rapid screening of Au (III) has been developed. Interestingly, the sensor could detect Au (III) in industrial waste with the presence of a common interference, Fe (III). As can be observed from Fig. 3, both electrochemical and colorimetric detection zones are patterned onto the paper substrate.

A three-dimensional microfluidic PAD for quantifying Ni, Cu, Fe, Pb, Cr, and Cd has been developed for contaminants that exhibit in airborne particulate matter (Rattanarat et al. 2013). Herein, colorimetric and electrochemical recognition on particular layers has been employed. The colorimetric detection methods are involved for the quantification of Cu, Ni, Fe, and total Cr. However, Pb and Cd are quantified electrochemically. Minimized cross-contamination and distinct reaction chemistry (e.g. agent masking, pH adjustments) are the added advantage of the reported three-dimensional microfluidic PAD with separate layers. This technique also enhances the analyte selectivity and sensitivity. Fabrication of a “lab-on-paper sensor” through inkjet printing with solgel entrapped β -galactosidase for heavy metal sensor by colorimetric visualization was reported. The developed paper assay was able to detect Hg, Ag, Cu, Cd, Pb, Cr, and Ni individually or in mix within 10 min (Hossain and Brennan 2011).

On the other hand, the vulnerability to numerous non-metal inorganic complexes has prompted environmental controls and approaches for building up allowable

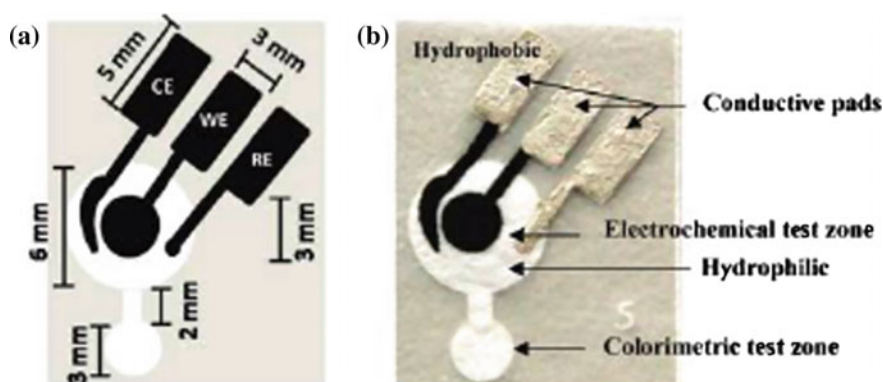


Fig. 3 Dual electrochemical and colorimetric paper sensor (Apilux et al. 2010)

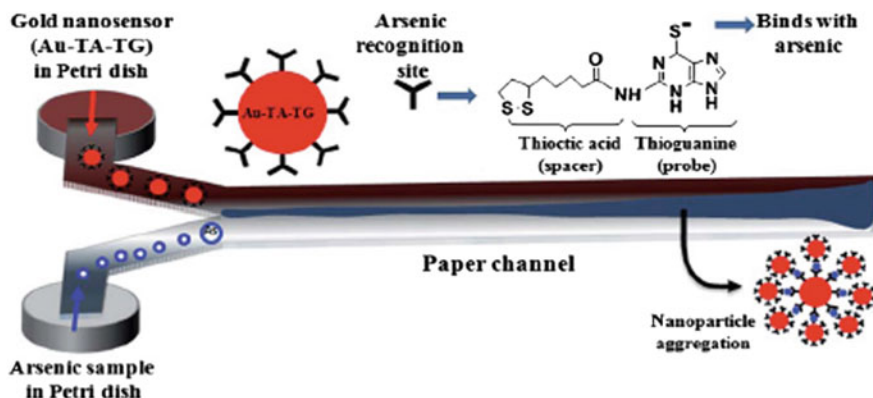


Fig. 4 Microfluidic PAD for arsenic detection (Nath et al. 2014)

exposure concentrations. The main pathway for inorganic pollutants into the environment is by combustion by-product or agricultural run-off or other specimen matrices from animal production facilities. Various low-cost microfluidic PADs have been reported for monitoring inorganic materials in the environment. Microfluidic PAD with colorimetric detection of phosphate, nitrate, nitrite, ammonia, arsenic, and cyanide has been reported and beautifully arranged in a review article by Henry Group (Meredith et al. 2016). Electrochemical recognition of iodide, bromide, chloride, potassium, and ammonium has been shown on a μ PAD utilizing potentiometry. Among these, Nirpen chanda et al. had successfully developed a rapid gold nanosensor for arsenic; the gold nanosensor was prepared by stepwise chemical conjugation of gold nanoparticles (AuNPs) with thioctic acid (TA) followed by thioguanine (TG) molecules. A visible bluish-black colour precipitate due to the formation of gold nanoparticle aggregates through transverse diffusive mixing of Au-TA-TG with As^{3+} ions on a paper device depicts the successful detection of arsenic (Nath et al. 2014) (Fig. 4).

Usual methods require an extra reader to quantify the concentration of target analytes in the sample, thus increasing the costs of using microfluidic PADs. However, a novel method for detection or readout has been developed recently. Interestingly, Lewis et al. reported a simple way to quantify the level of mercury and lead with respect to the “time” by noting from the “start” and “stop” (Lewis et al. 2014) (Fig. 5).

Jayawardane et al. proposed a paper device to test the presence of nitrite and nitrate in drinking water (Jayawardane et al. 2014) under optimal conditions and allowed the user to measure concentrations as low as 1 and 19 μ M for nitrite and nitrate. Recently, detection of CeO_2 or ceria nanoparticle through paper-based assay has been reported by employing redox-active ligands containing o-dihydroxy functionality, enabling multivalent binding, surface retention, and formation of charge transfer complexes between the grafted ligand and the ceria nanoparticles.

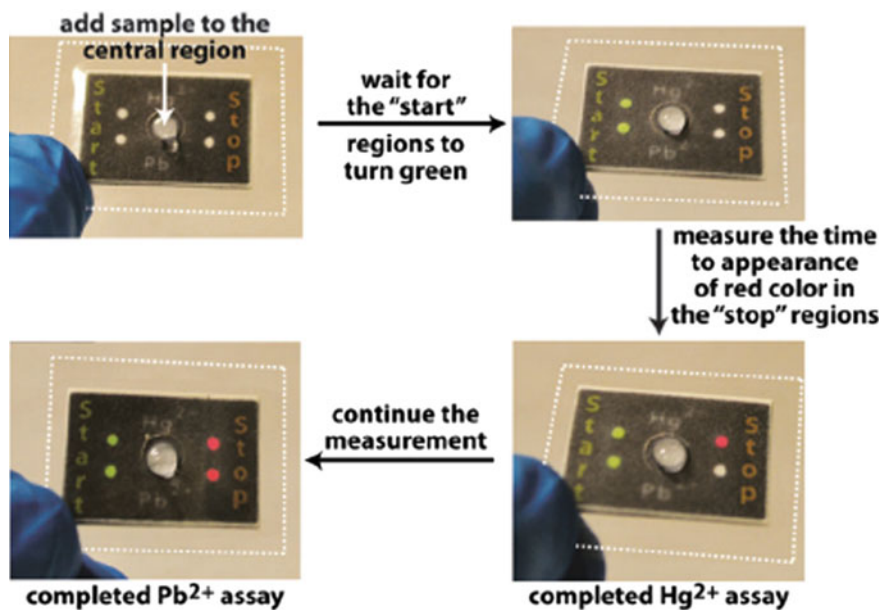


Fig. 5 Paper assay for the concurrent detection of mercury and lead (Lewis et al. 2014)

Using this strategy, paper-based and microarray-printed platforms with NP-capture ability involving either catechol or ascorbic acid as ligands were successfully fabricated (Othman et al. 2017).

3.2 Organic Molecules

Organic contaminants have various antagonistic health impacts relying upon the mechanism of activity or the affected organs. Because of their toxicological effects and hazard nature to human health, sensors to detect volatile organic compounds (VOCs) got much attention. However, most of the fabrication of sensors involves complicated procedures, and the need of additional equipment for detection is required. Hence, PAD-based sensors have been accounted for VOCs' recognition.

Yoon et al. (2013) reported inkjet-printed paper-based VOC sensor strips imaged with polydiacetylenes. Bisurethane-substituted diacetylene monomer, 5,7-dodecadiyne-1,12-diol bis[[(butoxycarbonyl) methyl]urethane] (4BCMU) have been inkjet printed on paper substrate, which under UV exposure displayed a blue colour and upon in the presence of chloroform, exhibited an yellow colour.

A reagent less, self-integrated paper assay with colorimetric method has been reported in 2015 for the detection of bisphenol A using the enzyme tyrosinase (Alkasir et al. 2015). Gas-separation microfluidic PADs without membranes are

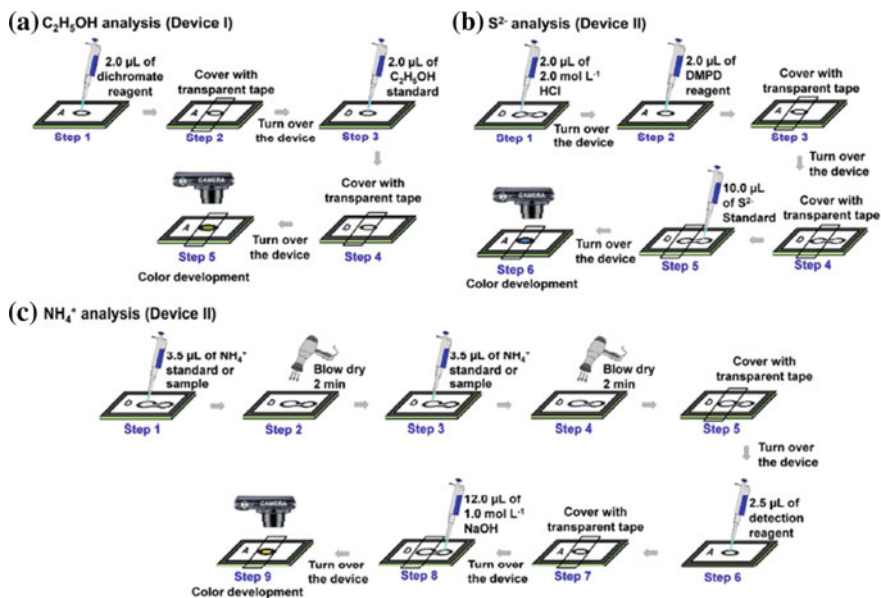


Fig. 6 MBL-GS μ PADs for **a** ethanol analysis, **b** sulphide analysis, and **c** ammonium analysis (Phansi et al. 2016)

utilized for quantification of all sorts of materials, and compounds have been reported, and in this case, the authors use different layers, three in number like: “spacer”, “donor”, and “acceptor layer”. The two layers, acceptor and donor, are composed of paper (filter) which has a pattern printed on it. These two layers are stacked one over the other using another layer called spacer in the middle of the two. Spacer layer is having a two-faced stacking tape, which is 0.8 mm thick, having a disc which is apparently small and is made open for the diffusion of the gas molecules from the donor layer to the acceptor layer (Fig. 6). Diffusion of the gas can be identified by the colour change which is resulted by the reaction of the gas molecules with the reagent which is present in the acceptor zone. The colour intensity is quantified by utilizing ImageJ software (Phansi et al. 2016).

3.3 Pesticides

Current advances in environmental paper analytical devices applications include the determination of toxic contaminants released or leftover from insecticides or pesticides. Air, water, soil, food, and feed products are always contaminated with pesticides. By means of ingestion, inhalation, and dermal assimilation, pesticide toxicity can happen and is related with neurotoxicity, hepatotoxicity, renal harmfulness, dermatitis, and cancer. Microfluidic PADs offer cost-effective, practical

methods for quickly examining food items for pesticides. Hossain et al. (2009) reported the detection of pesticides in beverage and food samples with a reagent-less bidirectional lateral flow bioactive paper sensor. The sensor consisted of acetyl cholinesterase (AChE) enzyme and indophenyl acetate reagent for the assay where the combination of the two created a blue colour. The pesticides presence was noticed by change in the blue colour intensity and analysed using a digital camera. The same group recently developed much more sensitive device using smartphone application for on-site quantification of colorimetric visual records to monitor organophosphate pesticides using the previously developed microfluidic PADs (Sicard et al. 2015). Recently by using nanoceria-coated paper device, simple, low-cost, and rapid detection of organophosphate (OP) pesticides employing an enzyme inhibition assay with AChE and choline oxidase (ChOX) was reported. When acetylcholine is present in the medium, AChE and ChOX will react and enhance the production of H_2O_2 and is identified colorimetrically using gadget which is coated by nanoceria, which will help in the development of colour (yellow). When it is allowed to react with OP pesticides, it can be seen that the activity of AChE was terminated, leading to the decreased production of H_2O_2 , and henceforth a strong decrease in the intensity of the yellow colour produced. This method of detection can be used to detect various OP pesticides such as, methyl-paraoxon and chlorpyrifos-oxon, and hence limits the use of highly established methods and equipments (Nouanthavong et al. 2016). Pentachlorophenol was detected on a microfluidic PAD by utilizing molecular imprinting technique along with photoelectrochemical (PEC) sensing platform was reported by Sun et al. Herein, gold and polypyrrole-functionalized ZnO nanoparticles were found to be the main key for detection (Sun et al. 2014). Colorimetric bioassay on paper for paraxon was proposed based on AChE-catalysed enlargement of gold nanoparticle co-entrapped with the enzyme in a solgel-based silica material (Luckham and Brennan 2010). Both the acetylthiocholine containing test solution and Au (III) salt were spotted on paper decorated previously with 3 nm gold nanoparticles. Hydrolysis of the enzyme substrate generates thiocholine, which further reduces the Au (III) onto gold nanoparticles, inducing the nanoparticles growth, which results in an increase in colour intensity. The colour change produced was correlated with enzyme inhibition by paraxon. A potable bioactive paper for the detection of the degradation products of organophosphorus pesticides with an LOD of 2.5 ppm of malathion was also reported by Kavruk et al. (2013).

3.4 Warfare

Explosives can be any unstable molecules which are organic and also are composed of oxygen and nitrogen atoms along with hydrogen and carbon; whereas hydrogen and oxygen are highly oxidizable compounds, and they play a role as fuels during explosion. A new method has been developed to detect, identify, and distinguish the major explosives like triacetone triperoxide (TATP), 4-amino-2-nitrophenol

(4A2NP), nitrobenzene (NB), picric acid, and hexamethylene triperoxide diamine (HMTD) utilizing paper-based devices, mobile phones, and chemometric equipments. Semi-quantitative quantification and analysis are also possible if the explosives are in less quantity, i.e. less than 0.2 μg . These paper-based sensors are incorporated with agents or chemicals, which will produce or assure a colour which is an important marker for the detection of each explosive. The colour pattern developed for each explosive can be quantified utilizing smartphones; software's developed to serve the purpose and also using a chamber which is closed to overcome the problems of illumination, which is an important limitation in commonly used paper sensors or gadgets (Salles et al. 2014). μPAD design has been developed for detecting 1,3,5-trinitrobenzene (TNB), 2,4,6-trinitrotoluene (TNT), and 2,4,6-trinitrophenylmethyl nitramine (tetryl) in explosive residue (Pesenti et al. 2014). Here, potassium peroxide acts as a complexing reagent, and analyte transfer occurs via a swab or by wiping a contaminated surface with the device. Violet colour is produced due to the reaction of methoxide or hydroxide ions (Janowski reaction) with trinitro aromatic compound. μPADs with five different lanes were fabricated and wax printed using filter paper (chromatography) to develop hydrophobic lanes which will be helpful in the identification of explosives like nitrite, nitrate, perchlorate, and chlorate oxidizers, also ammonium. Explosives used in military like RDX and TNT, and also strong explosives like nitrates and urea, are also detected using paper sensors. Paper sensors are also capable of detecting peroxide compounds like TATPs and the hydrogen peroxide precursors (Peters et al. 2015). Very recently, a paper sensors strips were also employed to quantify mustard gas concentrations, a chemical warfare agent. The detection is based on sulphur mustard gas decomposition in the presence of mediator haloalkane dehalogenase which is also followed by pH change in the local medium. The alteration in the pH is identified or detected by the use of pH strips, wherein the colour change is from bluish green to yellow, and the colour change is attained in 10 min (Bidmanova et al. 2016). A microfluidic PAD has been developed by Dubey et al. to detect the presence of vesicant and nerve agents. Here in this method, the procedure for detection of the compounds was relied on the mechanism of their action with *para*-nitrobenzyl pyridine and rhodamine hydroxamate, developing blue and red colours (Pardasani et al. 2012). A rapid and sensitive strip-based quick test for nerve agents like Tabun, Sarin, and Soman are detected rapidly utilizing strip-based devices incorporated with BODIPY-altered silica compounds and were reported by Climent et al. (2016) These strips were made up of spots of hybrid material, which act as an indicator, which consists of a fluorescent BODIPY compound covalently bonded to the lanes of mesoporous SBA silica particles. The change in the fluorescence or the fluorescence quenching will help in the sensitive and selective identification of chemical-based warfare agents.

4 Paper Analytical Devices for Biomedical Applications

In the last decade, paper-based microfluidics field has encountered fast development. Microfluidic PADs were initially created for point-of-care (POC) medicinal diagnostics in resource-poor scenarios and are presently connected to diverse territories. Paper sensors are cost effective and also demonstrate extraordinary guarantee for on-field analysis.

Biomedical applications going from medication, delivery and infection analysis to POC gadgets, and tissue designing have got expanding consideration in the recent decade. Regular biomedical strategies however frequently confront expanding challenges in various biomedical applications, for example, high cost, moderate determination, costly instrumentation, low drug delivery efficiency, and high disappointment rates in medication discovery because of the inconsistency between 2D cells-based assays and living tissues. Furthermore, many instances of worldwide maladies (e.g. intestinal sickness, tuberculosis, or TB, meningitis and hepatitis B) occur in high poverty zones, for example, rural areas and developing countries which regularly cannot manage the cost of costly and high-precise instruments. For example, as per World Health Organization (WHO), one million instances of bacterial meningitis are evaluated to happen and 200,000 loss of life in every year due to meningitis. All these pose great difficulties to regular biomedical strategies.

Long patient hold up and constrained options to analysis and treatment are currently common in health care sector, because of the interminable underfunding of human health care services. In this scenario, a low-cost POC for diagnostics purpose is need of the hour. Low-cost, versatile, and simple analytical devices could be the alternative for essential therapeutic and equipments accessible for underdeveloped countries (Yager et al. 2006). In addition, even the developing countries with built up healthcare insurance frameworks could also find benefits in POC-based diagnostics. Moreover, POC diagnostic devices for on-field utilization could find importance in military, forensic, and space operations. Paper-based POC offers a simple way for detection and are designed for single use and also can be disposed off easily. Hence they are widely used for point-of-care applications.

While considering the tremendous developments in PAD based diagnostics methods in the last decade, the most known strip based pregnancy test, have the benefits like effortlessness, biodegradability, and low cost for manufacturing and fabrication. Having said that, paper-based devices when compared with chip-based microfluidic devices, the later still linger behind when the logical affectability, multiplexing capacity, and shelf life issues are concerned (Yeo et al. 2011).

4.1 Applications of PADs in Medical Diagnostics

Health of an individual can be assessed by testing the biomarkers or the pathogens or the agents that are responsible for the deteriorating affects in health. Biomarkers could be nucleic acids, proteins, lipids, or metabolites. Biomarkers could be nucleic acids, proteins, lipids, or metabolites and are specific to particular diseases, which could be utilized for analyzing different diseases precisely (Li et al. 2002; Jin et al. 2003; Li and Jin 2002). For instance, to monitor and screen prostate disease, prostate specific antigen (PSA) has been generally used (Thompson et al. 2004). Ideally, the biomarker of interest for screening any disease should be exact, specific, and easily available for testing. In general, biomarkers are found in saliva, urine, serum, and tissue samples. However, more non-invasive samples are desirable for analysis. Paper-based devices, as specified some time recently, are a solid and cheap contender for detection of any clinically important analytes. Herein, some of the advances made in paper-based bioassays for human well-being diagnostics are discussed.

4.1.1 Detection of Nucleic Acids

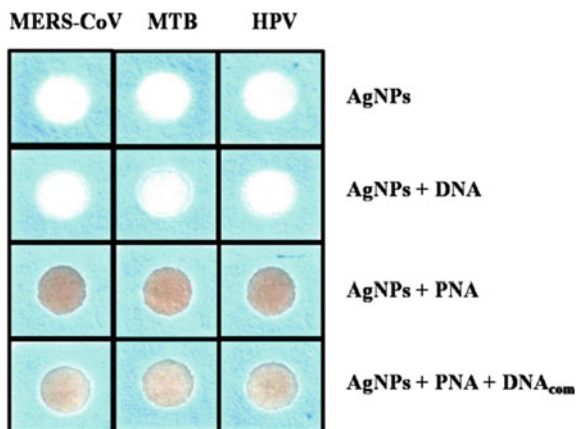
The detection of nucleic acid is important in various fields like, molecular biology, plant pathology, dairy, and medical diagnostics. It is also helpful in recognizing the aetiological agents of disease specifically with the specimens available from clinics that will help to monitor unculturable or picky pathogens. In addition, by utilizing amplified microbial DNA/RNA one can detect these disease making pathogens (Liu et al. 2011). With the intensification or amplification step, biomarkers based on nucleic acid in ultra-low levels could be detected (Poppert et al. 2005). As an inexpensive, compact, dispensable, and rapid nucleic acid identification technique, microfluidic PADs for nucleic acid hybridization have generated incredible enthusiasm in the scientist community.

Paper-based activated microfluidic device which was bound with Cy3-tagged test DNA which is single-stranded (ssDNA) has been used for fast recognition of the target by Araújo et al. By utilizing the cheap and promptly accessible connecting reagent which is bifunctional, 1,4-phenylenediisothiocyanate helps in the enactment of Whatman No. 1 filter paper, allowing it agreeable to form conjugation of Cy3-marked ssDNA. As a result of the inborn capillary action of the paper grid, which encourages fast specimen movement along the different test DNA, which in further helps the hybridization process to be completed within 2 min. The paper-based microfluidic strips can be used for fast, specific, and sensitive DNA detection in diagnosis and was also accomplished by the segregation of the amplified products produced from animal (dogs) and human genomic and mitochondrial DNA in forensics (Araújo et al. 2012). A low-cost paper-based microfluidic gadget has also been developed by Cunningham et al. for recognition of the oligonucleotide and protein. The detection was based on the “target-induced

conformational switching” of an aptamer connected to an electrochemically active reporter (Cunningham et al. 2014). A piece of paper combined with reverse transcription loop-mediated isothermal amplification for detecting dengue virus ribonucleic acid has been discovered in 2012. The colorimetric method gave a molecular level monitoring of dengue fever within a short time (60 min) while comparing to the conventional polymerase chain reaction techniques (Lo et al. 2012). A photoelectrochemical (PEC) device on a paper strategy has been utilized to selectively and sensitively detect or sense the presence of DNA, utilizing graphene-based permeable Au-paper as the working electrode for the photoelectrochemical reactions (Wang et al. 2013). Measurement component is depended on the ability of the novel paper supercapacitor (PS) to charge, which is built on a stage by produced photocurrent. A two distinctive supramolecular designs for impedance-based determination of DNA hybridization with respect to paper electrodes has been designed by Ihalainen et al. (2014). The primary design includes formation of self-assembled monolayer on gold nanoparticles decorated electrodes and further formation of streptavidin and biotin conjugated DNA. The second was based on the mixed thiol/DNA probe monolayer’s (Ihalainen et al. 2014). Further surface plasmon resonance and impedance parameters showed the apparent changes with respect to the DNA hybridization (Fig. 7).

Very recently, a paper-based colorimetric assay for DNA detection based on pyrrolidinyl peptide nucleic acid (apcPNA)-induced nanoparticle aggregations reported as an alternative to traditional colorimetric approaches. PNA probes are an attractive alternative to DNA and RNA probes because they are chemically and biologically stable, easily synthesized, and hybridize efficiently with the complementary DNA strands. The multiplex colorimetric PAD was developed for concurrent monitoring of DNA associated with diseases due to bacterial and viral infections, including Middle East Respiratory Syndrome Corona Virus (MERS-CoV), Mycobacterium tuberculosis (MTB), and human papillomavirus (HPV) (Teengam et al. 2017).

Fig. 7 Image of visual colour changes obtained from detection of MERS-CoV, MTB, and HPV in the presence of DNA (Teengam et al. 2017)



Regardless of the great execution of the paper-based nucleic acid hybridization for nucleic acid recognition, most of the time DNA hybridization depends on nucleic acid amplification process, which is often conducted off-chip and frequently requires costly massive hardware in the research facility. It is as yet difficult to coordinate DNA amplification ventures on paper-based devices because of the issues on fluid dissipation and heating component necessities.

4.1.2 Detection of Proteins

For early detection of a disease or preventions, it is imperative to analyse biomarkers. Mostly proteins also act as major biomarkers, important for research in medicine and medical diagnostics. The molecular biology techniques currently used are time-consuming to perform, expensive, require sophisticated instrumentation and also skilled persons are required to handle it. Hence, there is also an extraordinary need to manufacture or fabricate less expensive, fast, and basic POC detection methodologies with better feasibility and sensitivity, irrespective of resource-rich or less settings. Various paper-based microfluidic devices have been fabricated utilizing colorimetric, fluorescence, electrochemiluminescence, colorimetric, and electrochemical detection techniques for different clinically important biomarkers. Colorimetry-based immunoassay technique is a standout among the most generally utilized methods. The assay mostly depends on the colour change because of compound response between the bio-catalysts/catalyst connected to analyte of interest and the reagent added. The very well-known basic patterned paper device reported by Whitesides group beautifully explains the initial breakthroughs of microfluidic PADs for protein detection (Martinez et al. 2007). The authors used a known method of change in colour from yellow to blue while protein bind with tetrabromophenol blue (TBPB). The fabricated device exhibited the new possibilities available for the detection of bovine serum albumin in the clinically important concentrations. A paper-based microfluidic device to separate plasma from the blood comprised of blood partition film joined with patterned paper has been developed (Songjaroen et al. 2012). The whole plasma protein analysis through colorimetric assay further showed the greater efficiency of the method. It is worth to note that paper-based immunoassay utilizing polymerization-based amplification (PBA) for rapid monitoring of *Plasmodium falciparum* histidine-rich protein 2 (Pf HRP2) in malaria has been recently reported (Badu-Tawiah et al. 2015). Interestingly, the same group have tried another method for the signal amplification assessed performances of different amplification methods, like enzymatic amplification using HRP and alkaline phosphatase (ALP), PBA and also silver enhancement (SE) on AuNPs has been performed for PfHRP2 immunoassays on paper and its limit of detection (LOD) were found to be an order of magnitude lower than assays when compared with PBA or SE/AuNPs (Lathwal and Sikes 2016). 96-microzone paper-based device (P-ELISA) was reported by Whitesides group. The as developed device was easy to build and considering its biodegradable nature makes it more suitable than the common 96-well plates.

ELISA was performed utilizing alkaline phosphatase and BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate and nitrobluetetrazolium). Since the yellow colour BCIP/NBT reacts with enzyme and produces a colour change to purple, which can be easily visualized without any background colour interferences. The authors demonstrated the detection of rabbit IgG and specific antibodies from serums of HIV-1 positive patients. Even though the human serum samples were diluted to longer ranges, i.e. 10 times dilution, the test showed good results (Cheng et al. 2010). Human performance biomarker Neuropeptide Y detection was performed by using P-ELISA, and the authors were able to detect the analyte of interest in pico moles range (Murdock et al. 2013).

Sensitive and selective chemiluminescence ELISA (CL-ELISA) for the high throughput, rapid, stable, and reusable assay for cancer biomarkers were reported. Herein, chitosan and glutaraldehyde cross-linking were used for the entrapment of capture antibodies. The chemiluminescence was produced after the reaction between luminol-P-iodophenol and H_2O_2 with horseradish peroxidase (HRP)-marked antibodies. Paper-based CL-ELISA exhibited good detection limit for α -fetoprotein, CA-125, and CEA, (Wang et al. 2012). Colorimetric biosensing with respect to the colour change produced after the cross-linking of siloxane, 3-aminopropyltriethoxysilane, and glutaraldehyde onto paper was used for the monitoring of prostate-specific antigen (PSA). Herein, the authors immobilized anti-PSA antibody (Ab1) onto the paper surface and using GOx modified gold nanorod (GNR) as detection anti-PSA antibody (Ab2) label. The detection of PSA was achieved via the liberated H_2O_2 when the GOx label reacted with glucose. The colorimetric detection was based on the visual colour changes happened during the reaction of 3-aminopropyltriethoxysilane–glutaraldehyde complex reaction in presence of H_2O_2 (Zhou et al. 2014).

In general, most of the microfluidic PADs avail the washing procedure to completely remove the loosely binded to non-specific binding protein. But this technique is not so suitable in some cases hence it will interfere the assay procedure. To avoid these troubles, a novel washing procedure based on a ring-oven technique was proposed by Liu et al. Because of the capillary force and the continuous flow of washing solution, the non-specific binding proteins were moved to waste zone (ring area in Fig. 8a), thus making a low background for the detection (Liu et al. 2015). The authors further successfully showed the sensitive detection of carcinoembryonic antigen through TMB- H_2O_2 chemistry using the newly developed ring-oven washing technique (Fig. 8b).

Recently, hybrid microfluidic/paper microplate for ELISA has been reported. The microplate was fabricated by creating micro-wells in a poly(methyl methacrylate) (PMMA) chip and further insertion of wells with porous paper. The proof of concept was demonstrated by detecting immunoglobulin G (IgG) and hepatitis B surface antigen (HBsAg) in human serum samples. The paper/PMMA-based ELISA results could be observed with naked eye (Fig. 9). The porous paper in micro-wells not only helped in the successful immobilization of bio-reagents, but also avoided the tedious steps involved during the washing and modification procedure involved in the conventional ELISA (Sanjay et al. 2016).

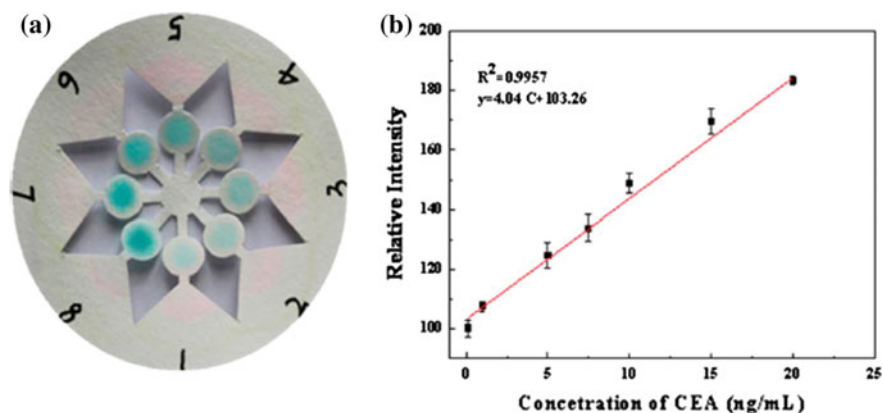


Fig. 8 a Image of visual colour changes obtained for different concentration of CEA and b corresponding calibration curve (Liu et al. 2015)

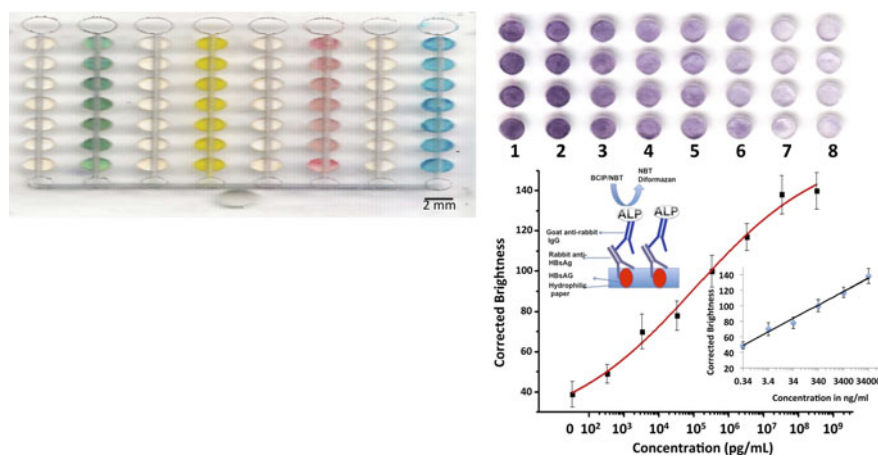


Fig. 9 Hybrid microfluidic microplate based on paper/PMMA chip for ELISA (Sanjay et al. 2016)

4.1.3 Detection of Cells

With improved specificity and affectability, sensing and diagnosis of tumour cells and other sorts of mammalian cells which are clinically significant have been materialized by using paper devices coupled with chemiluminescence, fluorescence, electrochemical, electrochemiluminescence, and colorimetric approaches. Thus, fabrication of inexpensive and simple, cheap microfluidic paper-based sensors for cell detection provides empowering opportunity to scale down the use of sophisticated instruments and also to increase the efficient point-of-care multiple screening methods.

By combining the techniques of electrochemistry and chemiluminescence in an origami principle based microfluidic PAD has been developed to analyze the tumour cells. The device consists of 3D macroporous Au conjugated with specific aptamers for the precise capture of cancer cells such as, MCF-7, HL-60, CCRF-CEM and K562 (Wu et al. 2015). Moreover the combination of a bimetallic gold and palladium nanoparticles decorated with concanavalin-A has been employed as a probe for cell recognition. The detection of human acute promyelocytic leukaemia cells and screening of anticancer drugs using a paper-based electrochemical device has been developed. The device is constructed with combination of gold nanoparticles-aptamer conjugates and enzyme catalysis onto paper device by employing origami and kirigami principles (Su et al. 2014). A differential pulse voltammogram (DPV) and fluorescence response gave a sensitive detection. Additionally, the same group employed the device they developed for the detection of K562 cells and to study the expression of glycans (Su et al. 2015). The Shevkoplyas group developed a microfluidic PAD for diagnosis of sickle-cell disease. This device enables differentiation of healthy patients from those carrying genes encoding for mutations in the structure of haemoglobin that lead to sickle-cell trait or sickle-cell disease. Lysing RBCs in a solution containing sickle dex caused structurally altered haemoglobin to polymerize, which, unlike normal haemoglobin, is insoluble and unable to be transported through paper by wicking. This approach provides a means of performing a diagnosis via visual inspection of the device (Yang et al. 2013). The impact of softwood and hardwood derived paper on microfluidic PAD for the mobility of red blood cells (RBCs) is reported by Li et al. The authors discovered that hardwood strands with low premise weight and higher porosity permitted the simple mobility of RBCs and indicated high clarity blood-typing assays. On the other hand, softwood filaments with more perplexing pore structure made RBCs mobility more troublesome and produced low clarity (Li et al. 2014). However, it is indeed worrisome that, without continuous perfusion, cell cultures based on paper devices can be only implemented by immersing the entire paper device in the cell culture medium and then applying nutrient or drug stimulation in sequence. Also, a technical drawback of creating a cell culture based on paper is the fluorescent interference caused by the paper fibre when both the cells and the paper fibres were stained. Nevertheless, multi-branched hybridization chain reaction (mHCR) and concanavalin-A (ConA) have been integrated for developing a colorimetric or fluorescence-based dual model paper-based device. Final product obtained due to mHCR process was altered to PtCu nanochains that were used as colorimetric signal, and the graphene quantum dot was utilized as fluorescence signal. These were used as such, unaltered, and were used potentially for analysing N-gleeman expression, which is a cell surface marker and can be used for analyzing the cells. These Au–Ag paper gadgets were used or enhanced the active sites and surface areas for accompanying aptamers in larger loads, and this strategy can further efficiently and specifically capture more cancer cells in larger amounts. In addition, the paper background fluorescence was effectively decreased by using this strategy. The device showed excellent fluorescence and colorimetric signals for the detection of breast cancer cell lines with a detection limit range between 25 and 35

cells per mL (Liang et al. 2016). In addition, bacteria cells were also detected by using paper analytical devices. A one-stage chromatographic paper-based test for the rapid sensing of *Salmonella typhi* in human serum utilizing gold nanoparticles was reported. The test was established through simple antigen and antibody interaction of *S. typhi* on nitrocellulose layer, and the authors then compared the test with respect to the dot blot assay and found that the immunochromatographic test is better and showed shorter time requirement for conducting the test. In addition, the paper test had longer shelf life (30 days) (Preechakasedkit et al. 2012).

4.1.4 Microorganisms

Recently much attention has been devoted for bacterial contamination detection particularly in food and water through microfluidic PADs with goals of providing diagnostic tools that are faster to answer and lower in cost than traditional methods such as culture analysis and polymerase chain reaction (PCR). In general, food-borne bacteria detection was accomplished by measuring the colour change occurring in a PAD, which is decorated with an enzyme associated with a pathogen of interest and further biochemical reaction. The chromogenic substrate used by the authors is β -galactosidase with chlorophenol red β -galactopyranoside (CPRG) for *Escherichia coli*; phosphatidylinositol-specific phospholipase C (PI-PLC) with 5-bromo-4-chloro-3-indolylmyo-inositol phosphate (X-InP) for *Listeria monocytogenes*; and esterase with 5-bromo-6-chloro-3-indolyl caprylate (magenta caprylate) for *Salmonella enteric* detection (Jokerst et al. 2012). Sensitive biosensing on a piece of paper was developed to detect of *E. coli* through nanoparticle aggregation strategy that was reported by Shafiee et al.; here, AuNPs were modified with lipopolysaccharide binding protein (LBP) to recognize bacteria (Shafiee et al. 2015). A hybrid microfluidic device with the combination of paper and polydimethylsiloxane coupled with loop-mediated isothermal amplification was reported by Dou et al. for the rapid, sensitive detection *Neisseria meningitides* (Dou et al. 2014). Smartphone-based detection of *Salmonella* was investigated by Yoon group by employing anti-*S. Typhimurium*-conjugated sub-microparticles modified paper for immunoagglutination and further scattering measurements (San Park et al. 2013). The whole detection process was done by using smartphone application, which helps to avoid the use of sophisticated instruments. In addition, while comparing with other smartphone based assay, here holder for the phone and sensor is not required. An economically feasible microfluidic PAD for *Salmonella* live cell detection based on quantification of adenosine triphosphate (ATP) has been developed (Jin et al. 2015); however, the technique is not selective since many other cell types such as mammal, plant, and yeast cells also release ATP. Rapid detection of influenza A H1N1 and H3N2 viruses was reported by Lei et al. By using a paper assay method, interestingly the same could be used for sub-typing also. By evaluating the internal structural protein, nucleoprotein (NP), and outer surface glycoproteins, H1 and H3, of the influenza viruses, a sensitive and specific test has been established through colorimetric sandwich immunoassay. The detection limits

for H1 virus were found to be 2.7×10^3 (particle forming units) pfu/assay and 2.7×10^4 pfu/assay for H3. The as developed test was able to detect the viruses in the clinical samples and infected cell lysates (Lei et al. 2015).

5 Conclusion

Since PADs are simple, cost effective, easy to handle and give on-site results, many paper sensors are fabricated for wide range of applications worldwide. These devices could be useful in rural areas and for developing countries where individuals find difficulty in bearing the cost of expensive instrumentation for diagnosis purpose as well as environmental monitoring. Researchers have shown new advantages that are obtained from paper-based hybrid microfluidic stages. Various identification systems such as colorimetric, fluorescence, chemiluminescence, ECL, and electrochemical location have been utilized as a part of microfluidic PADs investigation. Electrochemical discovery is additionally attractive for paper-based microfluidic devices, yet it is massive and costly. Albeit compact potentiostats are industrially accessible, the cost is still genuinely high. Colorimetric identification is exceptionally perfect with the way of ease to examine in resource-poor settings, yet affectability and quantitation are regularly bargained. In the most recent decades, advanced mobile phones have grown significantly. May the integration of technology and other electronic readers (e.g. glucometers) with PADs could give awesome effects on social insurance and ecological observing. For reliable PADs, it is essential to investigate the analytical performance under different test conditions (e.g. temperature, humidity, ambient light, complexity of the sample matrixes), perform stability studies (especially when sensitive reagents or materials are involved in the device architecture), and carry out interference studies.

Acknowledgements The authors are grateful to research grant from Department of Biotechnology, Government of India, and Yenepoya University.

References

- Alkadir RS, Rossner A, Andreescu S (2015) Portable colorimetric paper-based biosensing device for the assessment of bisphenol A in indoor dust. *Environ Sci Technol* 49(16):9889–9897
- Apilux A, Dunchai W, Siangproh W, Praphairaksit N, Henry CS, Chailapakul O (2010) Lab-on-paper with dual electrochemical/colorimetric detection for simultaneous determination of gold and iron. *Anal Chem* 82(5):1727–1732
- Araújo AC, Song Y, Lundeberg J, Ståhl PL, Brumer H III (2012) Activated paper surfaces for the rapid hybridization of DNA through capillary transport. *Anal Chem* 84(7):3311–3317
- Badu-Tawiah AK, Lathwal S, Kaastrup K, Al-Sayah M, Christodouleas DC, Smith BS, Whitesides GM, Sikes HD (2015) Polymerization-based signal amplification for paper-based immunoassays. *Lab Chip* 15(3):655–659

- Bidmanova S, Steiner MS, Stepan M, Vymazalova K, Gruber MA, Duerkop A, Damborsky J, Prokop Z, Wolfbeis OS (2016) Enzyme-based test strips for visual or photographic detection and quantitation of gaseous sulfur mustard. *Anal Chem* 88(11):6044–6049
- Bruzewicz DA, Reches M, Whitesides GM (2008) Low-cost printing of poly (dimethylsiloxane) barriers to define microchannels in paper. *Anal Chem* 80(9):3387–3392
- Carrilho E, Martinez AW, Whitesides GM (2009) Understanding wax printing: a simple micropatterning process for paper-based microfluidics. *Anal Chem* 81(16):7091–7095
- Cate DM, Adkins JA, Mettakoonpitak J, Henry CS (2014) Recent developments in paper-based microfluidic devices. *Anal Chem* 87(1):19–41
- Chen B, Kwong P, Gupta M (2013) Patterned fluoropolymer barriers for containment of organic solvents within paper-based microfluidic devices. *ACS Appl Mater Interfaces* 5(23):12701–12707
- Cheng CM, Martinez AW, Gong J, Mace CR, Phillips ST, Carrilho E, Mirica KA, Whitesides GM (2010) Paper-based ELISA. *Angew Chem Int Ed* 49(28):4771–4774
- Chitnis G, Ding Z, Chang CL, Savran CA, Ziaie B (2011) Laser-treated hydrophobic paper: an inexpensive microfluidic platform. *Lab Chip* 11(6):1161–1165
- Climent E, Biyikal M, Gawlitza K, Dropa T, Urban M, Costero AM, Martínez-Mañez R, Rurack K (2016) A rapid and sensitive strip-based quick test for nerve agents Tabun, Sarin, and Soman using BODIPY-modified silica materials. *Chem A Eur J* 22(32):11138–11142
- Cretich M, Sedin V, Damin F, Pelliccia M, Sola L, Chiari M (2010) Coating of nitrocellulose for colorimetric DNA microarrays. *Anal Biochem* 397(1):84–88
- Cunningham JC, Brenes NJ, Crooks RM (2014) Paper electrochemical device for detection of DNA and thrombin by target-induced conformational switching. *Anal Chem* 86(12):6166–6170
- Dornelas KL, Dossi N, Piccin E (2015) A simple method for patterning poly (dimethylsiloxane) barriers in paper using contact-printing with low-cost rubber stamps. *Anal Chim Acta* 858:82–90
- Dou M, Dominguez DC, Li X, Sanchez J, Scott G (2014) A versatile PDMS/paper hybrid microfluidic platform for sensitive infectious disease diagnosis. *Anal Chem* 86(15):7978–7986
- Dou M, Sanjay ST, Benhabib M, Xu F, Li X (2015) Low-cost bioanalysis on paper-based and its hybrid microfluidic platforms. *Talanta* 145:43–54
- Dungchai W, Chailapakul O, Henry CS (2011) A low-cost, simple, and rapid fabrication method for paper-based microfluidics using wax screen-printing. *Analyst* 136(1):77–82
- Fernandes SC, Walz JA, Wilson DJ, Brooks JC, Mace CR (2017) Beyond wicking: expanding the role of patterned paper as the foundation for an analytical platform. *Anal Chem* 89(11):5654–5664
- He Q, Ma C, Hu X, Chen H (2013) Method for fabrication of paper-based microfluidic devices by alkylsilane self-assembling and UV/O₃-patterning. *Anal Chem* 85(3):1327–1331
- He PJW, Katis IN, Eason RW, Sones CL (2015) Engineering fluidic delays in paper-based devices using laser direct-writing. *Lab Chip* 15(20):4054–4061
- Hossain SZ, Brennan JD (2011) β -galactosidase-based colorimetric paper sensor for determination of heavy metals. *Anal Chem* 83(22):8772–8778
- Hossain SZ, Luckham RE, McFadden MJ, Brennan JD (2009) Reagentless bidirectional lateral flow bioactive paper sensors for detection of pesticides in beverage and food samples. *Anal Chem* 81(21):9055–9064
- Hu J, Wang S, Wang L, Li F, Pingguan-Murphy B, Lu TJ, Xu F (2014) Advances in paper-based point-of-care diagnostics. *Biosens Bioelectron* 54:585–597
- Ihalainen P, Pettersson F, Pesonen M, Viitala T, Määttänen A, Österbacka R, Peltonen J (2014) An impedimetric study of DNA hybridization on paper-supported inkjet-printed gold electrodes. *Nanotechnology* 25(9):94
- Jahanshahi-Anbui S, Pennings K, Leung V, Kannan B, Brennan JD, Filipe CDM, Pelton RH (2015) Design rules for fluorocarbon-free omniphobic solvent barriers in paper-based devices. *ACS Appl Mater Interfaces* 7(45):25434–25440

- Jayawardane BM, Wei S, McKelvie ID, Kolev SD (2014) Microfluidic paper-based analytical device for the determination of nitrite and nitrate. *Anal Chem* 86(15):7274–7279
- Jiang X, Fan ZH (2016) Fabrication and operation of paper-based analytical devices. *Ann. Rev. Anal. Chem.* 9:203–222
- Jin W, Li X, Gao N (2003) Simultaneous determination of tryptophan and glutathione in individual rat hepatocytes by capillary zone electrophoresis with electrochemical detection at a carbon fiber bundle—Au/Hg dual electrode. *Anal Chem* 75(15):3859–3864
- Jin SQ, Guo SM, Zuo P, Ye BC (2015) A cost-effective Z-folding controlled liquid handling microfluidic paper analysis device for pathogen detection via ATP quantification. *Biosens Bioelectron* 63:379–383
- Jokerst JC, Adkins JA, Bisha B, Mentele MM, Goodridge LD, Henry CS (2012) Development of a paper-based analytical device for colorimetric detection of select foodborne pathogens. *Anal Chem* 84(6):2900–2907
- Kavruk M, Özalp VC, Öktem HA (2013) Portable bioactive paper-based sensor for quantification of pesticides. *J Anal Methods Chem* 2013:8
- Lathwal S, Sikes HD (2016) Assessment of colorimetric amplification methods in a paper-based immunoassay for diagnosis of malaria. *Lab Chip* 16(8):1374–1382
- Lei KF, Huang CH, Kuo RL, Chang CK, Chen KF, Tsao KC, Tsang NM (2015) Paper-based enzyme-free immunoassay for rapid detection and subtyping of influenza A H1N1 and H3N2 viruses. *Anal Chim Acta* 883:37–44
- Lewis GG, Robbins JS, Phillips ST (2014) A prototype point-of-use assay for measuring heavy metal contamination in water using time as a quantitative readout. *Chem Commun* 50(40):5352–5354
- Li XJ, Jin WR (2002) Monitoring homovanillic acid and vanillylmandelic acid in human urine by capillary electrophoresis with electrochemical detection. *Chin Chem Lett* 13(9):874–876
- Li X, Jin W, Weng Q (2002) Separation and determination of homovanillic acid and vanillylmandelic acid by capillary electrophoresis with electrochemical detection. *Anal Chim Acta* 461(1):123–130
- Li X, Tian J, Nguyen T, Shen W (2008) Paper-based microfluidic devices by plasma treatment. *Anal Chem* 80(23):9131–9134
- Li X, Tian J, Shen W (2010) Progress in patterned paper sizing for fabrication of paper-based microfluidic sensors. *Cellulose* 17(3):649–659
- Li L, Huang X, Liu W, Shen W (2014) Control performance of paper-based blood analysis devices through paper structure design. *ACS Appl Mater Interfaces* 6(23):21624–21631
- Liang L, Lan F, Li L, Ge S, Yu J, Ren N, Liu H, Yan M (2016) Paper analytical devices for dynamic evaluation of cell surface N-glycan expression via a bimodal biosensor based on multibranching hybridization chain reaction amplification. *Biosens Bioelectron* 86:756–763
- Liu P, Li X, Greenspoon SA, Scherer JR, Mathies RA (2011) Integrated DNA purification, PCR, sample cleanup, and capillary electrophoresis microchip for forensic human identification. *Lab Chip* 11(6):1041–1048
- Liu W, Cassano CL, Xu X, Fan ZH (2013) Laminated paper-based analytical devices (LPAD) with origami-enabled chemiluminescence immunoassay for cotinine detection in mouse serum. *Anal Chem* 85(21):10270–10276
- Liu W, Guo Y, Zhao M, Li H, Zhang Z (2015) Ring-oven washing technique integrated paper-based immunodevice for sensitive detection of cancer biomarker. *Anal Chem* 87(15):7951–7957
- Lo SJ, Yang SC, Yao DJ, Chen JH, Cheng CM (2012) Molecular-level dengue fever diagnostics: developing a combination of RT-LAMP and paper-based devices. *IEEE Nanotechnol Mag* 6(4):26–30
- Lu Y, Shi W, Qin J, Lin B (2009) Fabrication and characterization of paper-based microfluidics prepared in nitrocellulose membrane by wax printing. *Anal Chem* 82(1):329–335
- Luckham RE, Brennan JD (2010) Bioactive paper dipstick sensors for acetylcholinesterase inhibitors based on sol-gel/enzyme/gold nanoparticle composites. *Analyst* 135(8):2028–2035

- Martinez AW, Phillips ST, Butte MJ, Whitesides GM (2007) Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angew Chem Int Ed* 46(8):1318–1320
- Martinez AW, Phillips ST, Carrilho E, Thomas SW III, Sindi H, Whitesides GM (2008a) Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal Chem* 80(10):3699–3707
- Martinez AW, Phillips ST, Whitesides GM (2008b) Three-dimensional microfluidic devices fabricated in layered paper and tape. *Proc Natl Acad Sci* 105(50):19606–19611
- Martinez AW, Phillips ST, Whitesides GM, Carrilho E (2009) Diagnostics for the developing world: microfluidic paper-based analytical devices. *Anal Chem* 82(1):3–10
- Mentele MM, Cunningham J, Koehler K, Volckens J, Henry CS (2012) Microfluidic paper-based analytical device for particulate metals. *Anal Chem* 84(10):4474–4480
- Meredith NA, Quinn C, Cate DM, Reilly TH, Volckens J, Henry CS (2016) Paper-based analytical devices for environmental analysis. *Analyst* 141(6):1874–1887
- Müller RH, Clegg DL (1949) Automatic paper chromatography. *Anal Chem* 21(9):1123–1125
- Murdock RC, Shen L, Griffin DK, Kelley-Loughnane N, Papautsky I, Hagen JA (2013) Optimization of a paper-based ELISA for a human performance biomarker. *Anal Chem* 85(23):11634–11642
- Nath P, Arun RK, Chanda N (2014) A paper based microfluidic device for the detection of arsenic using a gold nanosensor. *RSC Adv* 4(103):59558–59561
- Nie Z, Nijhuis CA, Gong J, Chen X, Kumachev A, Martinez AW, Narovlyansky M, Whitesides GM (2010) Electrochemical sensing in paper-based microfluidic devices. *Lab Chip* 10(4):477–483
- Nouanthavong S, Nacapricha D, Henry CS, Sameenoi Y (2016) Pesticide analysis using nanoceria-coated paper-based devices as a detection platform. *Analyst* 141(5):1837–1846
- Olkkonen J, Lehtinen K, Erho T (2010) Flexographically printed fluidic structures in paper. *Anal Chem* 82(24):10246–10250
- Othman A, Andreescu D, Karunaratne DP, Babu SV, Andreescu S (2017) Functional paper-based platform for rapid capture and detection of CeO₂ nanoparticles. *ACS Appl Mater Interfaces* 9(14):12893–12905
- Pardasani D, Tak V, Purohit AK, Dubey DK (2012) μ -PADs for detection of chemical warfare agents. *Analyst* 137(23):5648–5653
- Pelton R (2009) Bioactive paper provides a low-cost platform for diagnostics. *TrAC Trends Anal Chem* 28(8):925–942
- Pesenti A, Taudte RV, McCord B, Doble P, Roux C, Blanes L (2014) Coupling paper-based microfluidics and lab on a chip technologies for confirmatory analysis of trinitro aromatic explosives. *Anal Chem* 86(10):4707–4714
- Peters KL, Corbin I, Kaufman LM, Zreibe K, Blanes L, McCord BR (2015) Simultaneous colorimetric detection of improvised explosive compounds using microfluidic paper-based analytical devices (μ PADs). *Anal Methods* 7(1):63–70
- Phansi P, Sumantakul S, Wongpakdee T, Fukana N, Ratanawimarnwong N, Sitanurak J, Nacapricha D (2016) Membraneless gas-separation microfluidic paper-based analytical devices for direct quantitation of volatile and nonvolatile compounds. *Anal Chem* 88(17):8749–8756
- Poppert S, Essig A, Stoehr B, Steingruber A, Wirths B, Juretschko S, Reischl U, Wellinghausen N (2005) Rapid diagnosis of bacterial meningitis by real-time PCR and fluorescence in situ hybridization. *J Clin Microbiol* 43(7):3390–3397
- Preechakasedkit P, Pinwattana K, Dungchai W, Siangproh W, Chaicumpa W, Tongtawe P, Chailapakul O (2012) Development of a one-step immunochromatographic strip test using gold nanoparticles for the rapid detection of *Salmonella typhi* in human serum. *Biosens Bioelectron* 31(1):562–566
- Rattananarat P, Dungchai W, Cate DM, Siangproh W, Volckens J, Chailapakul O, Henry CS (2013) A microfluidic paper-based analytical device for rapid quantification of particulate chromium. *Anal Chim Acta* 800:50–55
- Sackmann EK, Fulton AL, Beebe DJ (2014) The present and future role of microfluidics in biomedical research. *Nature* 507(7491):181–189

- Salentijn GI, Hamidon NN, Verpoorte E (2016) Solvent-dependent on/off valving using selectively permeable barriers in paper microfluidics. *Lab Chip* 16(6):1013–1021
- Salles MO, Meloni GN, de Araujo WR, Paixão TRLC (2014) Explosive colorimetric discrimination using a smartphone, paper device and chemometrical approach. *Anal Methods* 6(7):2047–2052
- Sameenoi Y, Nongkai PN, Nouanthavong S, Henry CS, Nacapricha D (2014) One-step polymer screen-printing for microfluidic paper-based analytical device (μ PAD) fabrication. *Analyst* 139(24):6580–6588
- San Park T, Li W, McCracken KE, Yoon JY (2013) Smartphone quantifies Salmonella from paper microfluidics. *Lab Chip* 13(24):4832–4840
- Sanjay ST, Dou M, Sun J, Li X (2016) A paper/polymer hybrid microfluidic microplate for rapid quantitative detection of multiple disease biomarkers. *Sci Rep* 6:30474
- Shafiee H, Asghar W, Inci F, Yuksekkaya M, Jahangir M, Zhang MH, Durmus NG, Gurkan UA, Kuritzkes DR, Demirci U (2015) Paper and flexible substrates as materials for biosensing platforms to detect multiple biotargets. *Sci Rep* 5:8719
- Sicard C, Glen C, Aubie B, Wallace D, Jahanshahi-Anbuhi S, Pennings K, Daigger GT, Pelton R, Brennan JD, Filipe CD (2015) Tools for water quality monitoring and mapping using paper-based sensors and cell phones. *Water Res* 70:360–369
- Songjaroen T, Dungchai W, Chailapakul O, Henry CS, Laiwattanapaisal W (2012) Blood separation on microfluidic paper-based analytical devices. *Lab Chip* 12(18):3392–3398
- Su M, Ge L, Ge S, Li N, Yu J, Yan M, Huang J (2014) Paper based electrochemical cyto-device for sensitive detection of cancer cells and in situ anticancer drug screening. *Anal Chim Acta* 847:1–9
- Su M, Ge L, Kong Q, Zheng X, Ge S, Li N, Yu J, Yan M (2015) Cyto-sensing in electrochemical lab-on-paper cyto-device for in-situ evaluation of multi-glycan expressions on cancer cells. *Biosens Bioelectron* 63:232–239
- Sun G, Wang P, Ge S, Ge L, Yu J, Yan M (2014) Photoelectrochemical sensor for pentachlorophenol on microfluidic paper-based analytical device based on the molecular imprinting technique. *Biosens Bioelectron* 56:97–103
- Taton TA, Mirkin CA, Letsinger RL (2000) Scanometric DNA array detection with nanoparticle probes. *Science* 289(5485):1757–1760
- Teengam P, Siangproh W, Tuantranont A, Vilaivan T, Chailapakul O, Henry CS (2017) Multiplex paper-based colorimetric DNA sensor using pyrrolidinyl peptide nucleic acid-induced AgNPs aggregation for detecting MERS-CoV, MTB, and HPV oligonucleotides. *Anal Chem* 89(10):5428–5435
- Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, Minasian LM, Ford LG, Lippman SM, Crawford ED, Crowley JJ (2004) Prevalence of prostate cancer among men with a prostate-specific antigen level ≤ 4.0 ng per milliliter. *N Engl J Med* 350(22):2239–2246
- Tomazelli Coltro WK, Cheng CM, Carrilho E, Jesus DP (2014) Recent advances in low-cost microfluidic platforms for diagnostic applications. *Electrophoresis* 35(16):2309–2324
- Wang S, Ge L, Song X, Yu J, Ge S, Huang J, Zeng F (2012) Paper-based chemiluminescence ELISA: lab-on-paper based on chitosan modified paper device and wax-screen-printing. *Biosens Bioelectron* 31(1):212–218
- Wang Y, Ge L, Wang P, Yan M, Ge S, Li N, Yu J, Huang J (2013) Photoelectrochemical lab-on-paper device equipped with a porous Au-paper electrode and fluidic delay-switch for sensitive detection of DNA hybridization. *Lab Chip* 13(19):3945–3955
- Wang J, Monton MRN, Zhang X, Filipe CD, Pelton R, Brennan JD (2014) Hydrophobic sol–gel channel patterning strategies for paper-based microfluidics. *Lab Chip* 14(4):691–695
- Wu L, Ma C, Ge L, Kong Q, Yan M, Ge S, Yu J (2015) Paper-based electrochemiluminescence origami cyto-device for multiple cancer cells detection using porous AuPd alloy as catalytically promoted nanolabels. *Biosens Bioelectron* 63:450–457
- Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH (2006) Microfluidic diagnostic technologies for global public health. *Nature* 442(7101):412–418

- Yagoda H (1937) Applications of confined spot tests in analytical chemistry: preliminary paper. *Ind Eng Chem Anal Ed* 9(2):79–82
- Yang X, Kanter J, Piety NZ, Benton MS, Vignes SM, Shevkoplyas SS (2013) A simple, rapid, low-cost diagnostic test for sickle cell disease. *Lab Chip* 13(8):1464–1467
- Yeo LY, Chang HC, Chan PP, Friend JR (2011) Microfluidic devices for bioapplications. *small* 7(1):12–48
- Yetisen AK, Akram MS, Lowe CR (2013) Paper based microfluidic point-of-care diagnostic devices. *Lab Chip* 13(12):2210–2251
- Yoon B, Park IS, Shin H, Park HJ, Lee CW, Kim JM (2013) A litmus-type colorimetric and fluorometric volatile organic compound sensor based on inkjet-printed polydiacetylenes on paper substrates. *Macromol Rapid Commun* 34(9):731–735
- Zhou M, Yang M, Zhou F (2014) Paper based colorimetric biosensing platform utilizing cross-linked siloxane as probe. *Biosens Bioelectron* 55:39–43