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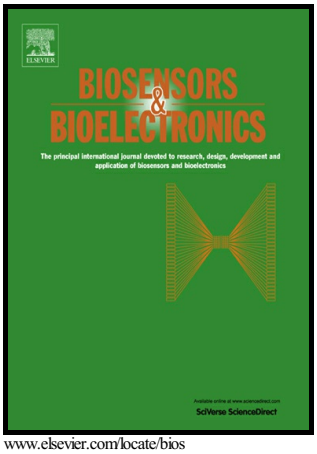

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Paper based diagnostics for personalized health care: Emerging technologies and commercial aspects

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Abstract:

Personalized health care (PHC) is being appreciated globally to combat clinical complexities underlying various metabolic or infectious disorders including diabetes, cardiovascular, communicable diseases *etc.* Effective diagnoses majorly depend on initial identification of the causes which are nowadays being practiced in disease-oriented approach, where personal health profile is often overlooked. The adoption of PHC has shown significantly improved diagnoses in various conditions including emergency, ambulatory, and remote area. PHC includes personalized health monitoring (PHM), which is its integral part and may provide valuable information's on various clinical conditions. In PHC, bio-fluids are analyzed using various diagnostic devices including lab based equipment and biosensors. Among all types of biosensing systems, paper based biosensors are commercially attracted due to its portability, easy availability, cheaper manufacturing cost, and transportability. Not only these, the various intrinsic properties of paper has facilitated the development of paper based miniaturized sensors, which has recently gained ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free, Deliverable to all end-users) status for point of care diagnosis in miniaturized settings. In this review, importance of paper based biosensors and their compatibility for affordable and low cost diagnostics has been elaborated with various examples. Limitations and strategies to overcome the challenges of paper biosensor have also been discussed. We have provided elaborated tables which describe the types, model specifications, sensing mechanisms, target biomarkers, and analytical performance of the paper biosensors with their respective applications in real sample matrices. Different commercial aspects of paper biosensor have also been explained using SWOT (Strength, Weakness, Opportunities, Threats) analysis.

Keywords: Paper based biosensors; Personalized health care; Point of care testing; Medical diagnosis; Biomarker; Real sample

1. Introduction

The contemporary world is suffering from most silent but griming health related issues. The World Health Organization has projected very serious statistics on the global health status (Shah, 2016). According to it, approximately one billion populations is deprived of health care services worldwide, and 36 million death counts are due to the non-communicable / communicable diseases which includes cardiovascular disorders, cancer, diabetes, chronic lung disorders, and malaria. This is almost two third of the estimated 56 million deaths each year worldwide (cardiovascular disease approximately 17 million; cancer about 7.6 million; chronic lung disease, about 4.2 million; diabetes about 1.3 million) (Shah, 2016). With the consideration of minimizing these severe cases, several nations have introduced impactful reformations in their public health policies across the globe. Nevertheless, The United Nations has also enacted a number of programs for improving the public health. Apart from this, numerous healthcare models have also been proposed by the leading agencies and policy makers (Obama, 2016). Although various improvements in the governmental policies have been fostering healthcare systems since long, numerous factors challenging its proper implementation and smooth progressions exists such as; the cost effectiveness of healthcare models, diagnostic kits, and services thereafter. In addition to these factors, technical competencies of various diagnostics play an important role in implementing technological advancements of healthcare products, commercial viability, and end-user acceptability. To promote health care services, various pharmaceutical companies, private healthcare bodies, and diagnostics manufacturer have actively participated in various campaigns. As a result, the world has witnessed various developments in portable health tracking, several disease monitoring systems, and dedicated health management models to foster PHC (Butts et al., 2013).

1.1. Personalized health care (PHC)

Progression of illness is contributed by various factors including genetic expressions and environmental exposures where consistent interplays of such factors can significantly alter the health status. Thus, by knowing one's fitness level, disease progression can be de-convoluted to design a proper therapeutic plan, which is also called as systems medicine (Hood, 2013). Currently, the clinical investigations are practiced where it starts from the complaints raised by patients which is followed by differential diagnoses (Snyderman, 2012). Although health care practices have become highly precise and contextual, yet it is incapable of addressing various multitudinous complexities of many diseases. PHC comprises of Personalized, Predictive, Preventive, and Participatory approaches also known as "P4

medicine", holds an important positions in modern diagnostics which is efficiently addressing various dynamic complications (Butts et al., 2013; Snyderman, 2012). Thus, PHC plays a crucial role in transforming conventional disease-oriented treatments to the personalized care where patients are diagnosed with customized assessments based on their clinical history and health profile. As per the PHC model, an effective diagnosis can only be done if the clinical conditions and complications of patient are well documented, which predominantly includes clinical history *viz.* past records of illness, infections, pathologic details *etc.*, and health profile details including body mass index and levels of various biomarkers in body fluids.

1.2. Point of care testing (POCT) devices for PHC

Modern day's technological advancements have shifted the paradigm of clinical diagnoses to the bio-molecular basis (Chandra, 2016). Practically, the detection of biomarkers in pre-symptomatic period are much reliable to rescue the patient. To achieve an efficient detection of such biomarkers various assays have been developed using lab based techniques. Nowadays, conventional detection methods are being challenged by kit based techniques, frequently called as biosensors (Chandra et al., 2015; Kashish et al., 2015). These essentially work based on the principle of molecular recognitions which enables whole process rapid and accurate (Chandra, 2016; Choudhary et al., 2016). For instance, Pallela *et al*. have demonstrated a bio-molecular detection tool that is capable of detecting metastatic cancer cells based on antibody and advanced nano-catalyst (Pallela et al., 2016). This work not only implies the detection of metastatic cells, but also provides the information of cell stages. Similarly, Thakur *et al*. have reported a highly sensitive electrochemical biosensor that can probe extracellular acidity by measuring pH that has various implications for cellular health monitoring (Thakur et al., 2015). Most recently, a unique dual amplification strategy has been employed to differentiate cancer cells by using the mDNA-aptamer-magnetic bead conjugate using calorimetric detection (Yu et al., 2016). These diagnostics have gradually evolved as biosensors from earliest type lab based cumbersome instruments to the bench-top or handheld devices for bio-molecular analyses (Chandra et al., 2010; Chandra et al., 2012; Maurya et al., 2016; Won et al., 2013; Yadav et al., 2013). Nowadays, biosensor research thrusts have been aimed to achieve the ASSURED status in all area of medical diagnostics. Extensive researches on value addition as well as development of new prototypes have also been done which has paved way towards development of lab on chip technologies; microfluidic paper based analytical devices (μPAD); and lateral flow assays (LFA). An adequate platform for various biosensors and diagnostics, paper has widely been appreciated for its potential applications in low cost settings. In continuation, the applicability of paper in various commercially viable diagnostic prototype fabrications for PHC, this review summarizes about existing personalized health monitoring devices with contrast to the paper based alternatives. Furthermore, it also summarizes the recent advancements on paper based POCT fostering PHC in order to achieve the goal of

wellness. An overview of PHC showing its various elements, features of conventional bench top/lab based diagnostics, and the paper based POCT device for PHM has been shown in **figure 1.**

1.3. Current trends in POCT devices:

The POCT refers to onsite rapid diagnosis in miniaturized module providing greater sensitivity and selectivity, which plays an important role in PHM and can be performed even by untrained personnel (Gubala et al., 2012). The demand of onsite prompt diagnostics has become high due to the long queues of test takers derived by constraints on insufficient infrastructures or shortage of manpower in nodal and centralized laboratories. POCT assist in producing rapid, accurate, and quantitative test results that provide ease to both clinicians as well as to patients. So far, commercial POCT devices have been used to detect glucose, pregnancy, malaria, and typhoid analyzers (Gubala et al., 2012; Liu et al., 2007), however, several POCT prototypes have also been established such as; detection kits of uric acid (Kumar et al., 2015a), glutathione (Noh et al., 2012), lactate (Rassaei et al., 2014), creatinine (Zhybak et al., 2016), antibiotic concentrations in body fluids (Agrawal et al., 2013; Chandra et al., 2011; Zhu et al., 2012b) *etc*. In addition to these, POCTs have found several targets *viz*. biomarkers for disease condition (Rohrman et al., 2012), cancer cells (Pallela et al., 2016; Zhu et al., 2012a), extracellular nucleic acids (Oblath et al., 2013; Wen et al., 2016) *etc*.

So far, we have realized that various genre of POCT devices are constantly assisting PHM where the continuous monitoring of the biomarkers from body fluids and tissues are done. The technological advancement has consistently added great impact of PHM on the healthcare systems and planning. Having emphasized on the research and development on such domains many healthcare companies have targeted to produce diagnostic devices for PHM with the highest degree of precision by incorporating the state of art technologies. In a commercial overview, a number of diagnostic volumes have been marketed including iSTAT's (now acquired by Abbott) total blood analyzer, that quantifies the presence of wide range of components in blood. Similar products for blood analysis have also been launched by Epocal (acquired by Alere in 2010) and Abaxis with their own novelty (Chin et al., 2012). Some other renowned biosensor based POCT devices have also witnessed enormous commercial success in global markets are blood chemistries profiler (Biosurfit, Molecular Vision *etc.*), cardiovascular biomarker detection kit (Atonomics, Biosite (now Alere), LabNow, Molacular Vision, Nanomix *etc.*), cancer biomarker immunosensors (Cepheid, Genefluidics, LabNow *etc.*), HIV detection kit (Advance liquid logic, Alere, IQuum *etc.*), and biosensor for respiratory infections diagnosis (Cepheid) (Chin et al., 2012). **Figure 2** shows commercially available handy POCT devices being used for PHM. Although, these volumes are

immensely capable of biomolecular analyses, still they are not accessible to everyone due to several reasons. In order to mitigate the constraints related to accessibility, researcher have been inclined to design commercially viable, efficient, precise, and affordable alternatives using state-of-the-art technologies. Additionally, the post adoption of these commercially available POCT devices, end-users demand has been shifted towards cheap and robust paper biosensors. In a report on market analysis presented by "Grand View Research, USA", the current market share of such paper based diagnostics is approximately $2.2 billion which will reach to $8.35 billion by 2022 (Websource, 2016).

2. Paper as a compatible platform for low cost biosensors

Paper is a cheap, easy-accessible, soft matrix containing numerous capillaries facilitating the self-pumping of the fluid when comes in direct contact. Due to such properties, paper has become an alternative to the self-powered microfluidics for next generation personalized biosensing. For the first time, paper was used as diagnostic platform by Martin and Synge to access chromatographic techniques which were recognized for the Nobel Prize in 1952. Since then paper has been used for facile and robust platform for various analytical and bio-analytical techniques.

2.1. Properties of paper as a potential substrate for low cost microfluidics

Commercial manufacturing of paper sheets are practiced by most abundant plant materials *viz.* wood, cotton, jute, flax, hemp, bamboo, ramie, sisal, bassage *etc.* depending on the desired qualities and grades (Roberts, 1996). Commonly used wood made paper contains a chromophoric intrinsic compound lignin making it unsuitable for spectral characterizations of paper based diagnostic devices. Nevertheless, lignin content is much likely to get degraded in down-stream processing, but presence even in trace amount can significantly alter the performances of optical paper based diagnostics. Hence, higher grade lignin-less cotton-made chromatographic filter papers are preferred for this purpose (Academy, 2016). In addition to this, several other grades are also being utilized which are in general made by subtle modification of cellulose fibers with nitro compounds (Company, 2016). Such modifications have been introduced by acid treatments to the cellulosic fibers to add various mechanical and chemical properties to the paper (Britannica, 2016; Company, 2016). The intrinsic material properties of paper *viz.* surface characteristics, surface area, capillary flow rate, pore size, porosity, and thickness play an important role in deciding the fluid flow through paper based platforms. Unfortunately, there are no single grade paper is available where all desirable parameters can be controlled, hence, the selection of paper grade mainly depends on the analytical applications. The high porosity of paper matrix facilitate uncontrolled diffusion resulting the unconstrained wicking of liquids throughout the platform (Zhong et al., 2012) and the total effective

surface area of paper platform plays a crucial role in reagent deposition, sensitivity, specificity and reproducibility in biosensors. Moreover, paper grades are indexed in different numbers depending on the ratio of surface areas where usual categories lies in between 50-200 (index) are acceptable for biosensing applications (Millipore, 2013). In addition to this, capillarity is another parameter which shows the self-wicking capability of paper matrix when fluid comes in its contact. Since, the movement of fluids decays in exponential manner, capillary flow time has been considered for the further evaluation as it exhibit the linear relation with the capillary flow rate (Millipore, 2013).

Several other parameters also play the significant roles in the performance of paper based POCT devices. A statistical parameter for pore size estimation called as pore size distribution helps to govern the interrelatedness of capillary flow rate with the pores in matrix, which is also used for commercial categorization the paper into various grades (Doty et al., 2012; Yetisen et al., 2013). Moreover, the thickness of paper matrix helps in arresting bed volume, maintaining tensile strength, and enhancing signal visibility in colorimetric biosensing. Equation 1 shows an empirical relation between percent visibility and thickness of paper matrix. In addition to this, bed volume of paper matrix also play a crucial role in signal visibility, where greater volume introduces dull signal and lesser the bed volume makes it delicate in various aspects. Thus, it indirectly influences the signal strength in various paper based colorimetric detections (Millipore, 2013).

$$\%\ \boldsymbol{visible\ signal} = \boldsymbol{visible\ depth}\ (\mu) / \boldsymbol{membrane\ thickness}(\mu) \quad \textbf{(1)}$$

In context to manufacturing processes, the implication of this empirical relationship can be described as; paper of less thickness is more prone to have defects during major manufacturing processes, however, it produces better visibility due to its low bed volume. On other hand, the thicker sheet of paper introduces greater ease for manufacturing processes but visibility in diagnostics reduces due to lager bed volume. Thus, the thickness optimization of paper matrix becomes a great challenge in front of commercial production of paper based colorimetric biosensors. However, various complexities in bioassays have also been adopted by modified paper based matrices that have essentially crafted by various micro-fabrication techniques. Depending on the complexities of fluid handling and precision, so far paper based biosensors are broadly categorized under dipstick, LFA, and µPAD.

2.2. Types of paper based biosensors

As stated earlier in above sections, the complexity of the reaction mechanisms of detection is the major concern in papers based microfluidic devices. Various prototypes have been proposed providing the handling capacity ranging from simplistic type self-wicking to the barrier guided handling of fluid in complex microfluidic chip. Such complexities have led the categorization of the paper based biosensors

in to various genre including the dipsticks, LFA, and μ-PADs which are described in this section. In addition to these, the advantages and limitations, associated to these prototypes have been described in Table 1 along with various commercially available volumes. Few of the recent biosensing technologies are shown in Supplementary Information SI-Figure 1.

2.2.1. Dipstick type paper based biosensor

Dipstick biosensors are a simplistic diagnostic device which contains pre-deposited reagents and provide qualitative detection. Several examples are reported till now for the dipstick sensors *viz.* pH strips, uric acid strips, pathogen detection strips, glutathione strips *etc* (Nurul Najian et al., 2016; Singh et al., 2016). They are easy to fabricate and have great ease to commercial productions. However, they greatly suffer due to their inability to provide quantitative informations. Otherwise, when it comes to qualitative detection, where exact concentration of analyte is not so important to be informed, dipstick format fits the best for rapid and authenticated results. Figure 3(a) shows a typical colorimetric dipstick assay where the chromogenic molecule changes its oxidation state providing the visual color change (Kumar et al., 2015a).

2.2.2. Paper based Lateral flow assay (LFA)

Paper based LFA are advanced version of dipstick, which offers controlled fluid flow. These improvements in the test strips make them suitable for introducing complex designs for multistep assays. Particularly these LFA strips based prototypes are made up of the four integral parts as follows; sample pad (cellulose; filters the impurities and storage for the dried buffer), conjugation pad (glass fiber; dry reagent storage), detection pad (nitrocellulose; captures reagent and development of signals), and absorbent pad (cellulose fibres; provides the driving force for flow). Figure 3(b) shows the sensing mechanism and classical fluid flow from one zone to other in LFA assay for detection of prostate specific antigen (PSA) (Damborsky et al., 2016). Although the higher complexity level have been achieved in LFA strips, the limitations of quantification is still remained in this systems (Posthuma-Trumpie et al., 2009). In this format, the flow of reagents and samples merge in conjugation pad initiating the reactions which eventually forms visible band in the presence of analyte (Bahadır and Sezgintürk, 2016; InnovaBiosciences, 2016). Apart from this, LFA also gives the better sample handling followed by preprocessing of the sample to screen out interfering molecules.

2.2.3. Microfluidic paper based analytical device (μ-PAD)

The interventions of microfluidics based technologies have uncovered various hidden potentials of the paper based devices to strengthen the various limitations associated to LFA. μ-PAD not only added

several flow advantages, but also enabled multiplexing and quantitative analysis (Rezk et al., 2012). So far, several variant of paper based microfluidic devices have been reported where analysis can be done using chemiluminescence (Shi et al., 2012; Wang et al., 2012b), electrochemical (Nie et al., 2010), spectroscopic (Ellerbee et al., 2009), and peizo-resistive micro-electro mechanical system (MEMS) (Liu et al., 2011) based sensing. Furthermore, the colorimetric biosensing mechanisms have been supported by various paper based biosensors mainly provides two methods which falls under enzyme mimetic systems or enzyme assisted systems. These enzyme assisted systems are known for their high specificity and sensitivity, whereas enzyme mimetic systems show high sensitivity on a wide range of temperature. An illustrative sensing based on μ-PAD for enzymatic detection of beta-hydroxybutyrate has been shown in figure 3(c) (Wang et al., 2016). Other than this, number of biosensors have also been reported for detecting various analytes using body fluids *viz.* glucose, uric acid, and salivary analysis (Fischer et al., 2016; Nery and Kubota, 2016; Soni and Jha, 2015; Yin et al., 2015; Yu et al., 2011b). A paper based enzyme linked immunosorbent assay with comparative sensitivity has been reported by Cheng *et al*. for the detection of rabbit IgG with DL of 54 fM. In addition to this, same group has also described the enhanced performance of the μ-PAD by reducing level of paper borne interfering agents (Cheng et al., 2010). In another report, Dias an co-workers have reported a paper based glucose biosensor where glucose oxidase (GOx) was attached onto the paper offering an excellent limit of detection of 0.11 mmol L^{-1} (Dias et al., 2016). Enzyme assisted paper biosensors are powerful, however, they suffer due to the thermal stability and require special storage conditions. These issues have been resolved using enzyme mimetic systems based on biofunctionalized nanoparticles, quantum dots *etc* (Chandra, 2016). Furthermore, nanoparticle based enzyme mimetic strategies have also employed for the colorimetric detection in μ-PAD. These μ-PAD are not only limited to colorimetric detection, but it has also been successfully applied for detection of various analytes using chemiluminescence (Ge et al., 2014) and electrochemical techniques (Dungchai et al., 2009).

2.2.4. Smart accessories based paper bio-analyzer

The paper based biosensors are supported by various smart devices such as; smart phone, strip readers, dedicated electronic signal processing modules *etc*., for quantitative or semi quantitative estimation (Carvalhal et al., 2010). For instance, Martinez *et al*. have reported the peripherals aided high quality low cost diagnostic device (Martinez et al., 2008a). Among all paper based smart devices, usage of smartphones are leading over others due to their easy handling, tunability, and simplicity for the end-users. Moreover, the first time investment to those diagnostic kits are negligible as the end user is already accustomed with one or more smart-phone in present global scenario. Thus, the research impact of smart phone assisted paper based POCT have been encouraged. Recently, several schemes have been developed

using these smartphones such as; smartphones assisted LFA analyser (You et al., 2013), electrochemical sensing (Wang et al., 2015), and optical detections. Soni and Jha have reported a smartphone based paper biosensor for glucose detection from saliva where the colorimetric readout have been analyzed with the smartphone based image processing module (Soni and Jha, 2015). A simple paper based colorimetric device assisted by smartphone have been shown in figure 3(d) where the prototype was used for bacterial detection (Shafiee et al., 2015) The major advantages of using these systems are that these produces a rigid and authenticated organized records in cheaper cost for future referencing. Due to these potentials of the smartphone assisted paper based biosensing modules, commercially several cooperations have begun to establish such models with the help of leading smartphone manufactures and healthcare giants. As a result, there is an overwhelming emergence of smartphone based diagnostic devices for general healthcare and fitness (Vashist et al., 2016). Although, various prototypes have been proposed using smartphone assisted paper biosensor, unfortunately none of them have gained the full flagged commercialized status till date. Therefore, more work can be done towards development precise smart phone based sensor having ability to be commercialized.

2.3. Manufacturing and commercial feasibility of paper based diagnostics

Fabrication of these devices involves much straight-forward methods, usually practiced with 1D or 2D micro-fabrication as well as other advance techniques such as; photolithography (Martinez et al., 2007b; Martinez et al., 2008b), wax printing (Carrilho et al., 2009), etching , cutting (Kumar et al., 2015a), laser assisted (Kumar et al., 2015a), and 3D techniques (Fischer et al., 2016; Wu et al., 2015). Most of the technologies used for manufacturing of paper based biosensors comprises sealing of the pores, 2D cutting, shaping, wax patterning, flexographic printing, alkyl ketene dimer (AKD) printing *etc* (Dungchai et al., 2011; Songjaroen et al., 2012; Yetisen et al., 2013). Apart from these, the micro-channels have also been introduced in the paper based platforms using various existing precision technologies *viz.* cutting, photolithography, plasma etching, polydimethylsiloxane (PDMS) plotting, and wax printing (Dungchai et al., 2011). Furthermore, the specificity has been achieved by using biomolecules immobilized on the cellulose matrix of paper by various techniques such as; entrapment, adsorption, covalent attachments *etc*. For instance, Cradaou and Berthelot have reported a strategy to immobilize the enzyme molecule on paper based platform where carbon atom (C3) of glucose moieties of cellulose has been used for covalent attachment of enzyme molecules. Similarly, Karra-Châabouni *et al.* have reported an oxidation based approach with different carbon atom (C6) of glucose moieties in cellulosic backbone by using sodium bromide, sodium hypoclorate and 2,2,6,6-tetramethyl-piperidin-1-yloxyl free radicals (Credou and Berthelot, 2014; Karra-Châabouni et al., 2008). In another immobilization strategy, Ibrahim *et al.* have demonstrated an enzyme immobilization *via.* carboxylic group attachment to the cellulosic chains assisted

by subsequent treatment of sodium hydroxide and monochloroacetic acid (Ibrahim et al., 2013). Moreover, several immobilization methods have also been adopted using paper nanomaterial recombinant (Bodelón et al., 2014), azo dye (Isaad and El Achari, 2011), strong acid treatment (Kennedy and Quinton, 2000), cationic polymer mixture (Geffroy et al., 2000), sol gel entrapment (Hossain and Brennan, 2011), polymer layer enzyme sandwich (Wang et al., 2014) mediated techniques. So far, paper based biosensors for various diseases, pollutant, and clinically important compounds have been reported including cancer, HIV, malaria, typhoid, hCG *etc.* Some of these biosensors have successfully managed to penetrate into the healthcare market worldwide *viz.* malaria testing kit (SD BIOLINE Malaria Ag P.f/Pan, Standard Diagnostics Inc. Giheung-gu ,Korea; IMMUNOQUICK® Malaria falciparum, Biosynex Strasbourg, France), pregnancy strips (Accu-Tell ® Rapid hCG, Accu BioTech Co. Ltd. Newark, USA), HIV (Uni-Gold™ HIV, Trinity biotech manufacturing Ltd. Co Wicklow, Ireland), and typhoid kits (Multi-Test Dip-S-Ticks, PANBIO INDX, Inc.; TUBEX, IDL Biotech; TyphiDot, Malaysian Biodiagnostic Research SDN BHD).

The commercial feasibility of these biosensors depends on cost effectiveness and balance between the cost invested and return for unit volume, which can be introduced by multiplexing of such platform. The conventional work on multiplexing using test strip have been introduced by Free and coworkers which has been used for the simultaneous detection of the protein, glucose, and pH (Free et al., 1960). In another example, Wu *et al.* reported a three dimensional wax printed origami type paper based sensor for multiplex immunosensing (Wu et al., 2015). Most recent advancements in multiplexed detection have been introduced by Boonyasit and coworkers for differential detection of the total and glycated hemoglobin (Boonyasit et al.). Another multiplex biosensors has also been developed by Zhao *et al*., for the simultaneous detections of glucose, lactate, and uric acid in an electrochemical array (Zhao et al., 2013)**.** Ma *et al*. have developed the signal amplified photo-electrochemical cascade based immunosensor to detect the carcinoembryonic antigen (CEA) (Ma et al., 2016). Down the evolutionary trends, paper based biosensors found various strategies for the robust and ultrasensitive volumes, still they have limitation of shorter shelf life and transportation feasibilities (Ahmed et al., 2016). In order to give better shelf life, protein based bio-recognition elements are immobilized into the polymeric gels. For example, dextran based protectant have been used in paper based immunosensor showing shelf life upto 4 months (Cao et al., 2017). In addition to this, several strategies to replace the temperature sensitive moieties have also been adopted using aptamers. The LR and DL were the key issues in paper based diagnostics in recent past. So far, enormous efforts have been given to improvise the analytical performances of such volumes. For example, Lei *et al*. have proposed an excellent quantitative determination method of biotin-avidin interaction using carbon nanotube bundles on paper with a limit of detection of 25ng mL^{-1} (Lei et al., 2015). Apart from these ultrasensitive prototypes, various research groups have developed low cost

paper biosensors. For instance, Cinti and co-workers have developed an office paper based alcohol sensor with unprecedented DL of 0.52 mM (Cinti et al., 2017a). Paper based diagnostic volumes have several advantages over others, still there are several limitations and challenges. An analysis comprising of various assessments on the performance parameters and external factors for the commercialization of any product, also called SWOT (Strengths, weaknesses, Opportunities, and Threats) evaluates commercial success. **Figure 4** describes the SWOT analysis of paper based biosensing products that may greatly help to add values in fabrication and their translation.

3. Limitations and strategies to overcome the challenges of paper biosensor

Paper based devices possess enormous capabilities to detect various analytes in clinical complexities, but in cases where analytes are found in ultralow concentrations such as; amyotrophic lateral sclerosis, spinal muscular atrophies, myasthenic syndromes, and neuropathies (Scotton et al., 2014) paper based devices are not the method of choice. In order to build devices of greater efficiency and efficacy, paper based bio-analytical platforms should be improved. Practically encountered limitations associated with paper based platforms can be categorized as follows:

1. Inefficient sample flow through these devices
2. Inadequate fabrication technologies
3. Biosensing performance parameters

In paper biosensors, the volume of fluid transfer is dependent on various grades of paper. Since paper absorbs the fluid and spread it uniformly throughout on paper bed, causes insufficiency of sample in the special case of ultralow concentration of analytes (Li et al., 2012b). It has been found that, the fraction of sample feed volume reaches to the zone of detection is less than half in various commercial paper based strips due to the presence of water absorbing soaking pad that is essentially required for driving the sample towards detection zone. Consequently, it demands more sample amount which can increase the burden of sample collection process prior to detections. The proper and paper friendly fabrication technologies are limited to fewer expensive techniques, making the fabrication process highly dependent on high-end sophisticated equipments. Thus, the commercial fabrication processes are considered to be expensive and lengthy which also require trained personals. Apart from these, the selection and availability of materials for further modifications also play a crucial role. For instance, in the fabrication of micro-channels on paper, hydrophobic barriers are generally introduced using wax or AKD where these materials show hydrophobic properties facilitating a barrier for liquid penetration only in the case of liquid having surface tension greater than a critical value. In biological samples, where surfactants are

radially used due to which surface tension becomes lower than critical value, which makes the barrier prone for leakage resulting in short-circuiting of the micro-channels. Although, various limiting parameters such as; substrate spatial distribution, pore size distribution, microfluidic flow properties *etc.* have extensively been improved for the paper based biosensing platform, various commercially potent prototypes are still emerging. That imparts the further improvements in such prototypes in order to minimize the signal to noise ratio and detection time in all formats of paper based diagnostics (Ahmed et al., 2016) to compete with conventional POCT devices. Following sections will describe various strategies adopted to improvise such signals in various paper based platforms.

3.1. Signal improvement by modifications of paper substrate

The ratio between signals to noise plays a crucial role in the bio-recognition processes. In paper based detections such signals are recorded either by amplifying the signal strengths or reducing the surround noises. Owing to the bio-polymeric matrix, properties of paper sheets are highly random in spatial arrangements and thus show low accuracy. So far, several attempts have been taken for the improvements of performances and veracity of signal. Apart from these several manipulations of paper based platforms have been incorporated by tuning the fluid handling properties in the paper matrices. A number of theoretical and practical studies have also been done elsewhere indicating the importance of paper for ultrasensitive detection of analytes (Ahmed et al., 2016; Xia et al., 2016).

3.1.1. Improvement based on paper composition enrichment

The major constituents of paper includes, cellulose, hemicellulose are non-conducting insulators, usually, inhibits electrical signals making unfavorable for the electrochemical biosensing applications. Possibly, because of this reason, paper based biosensor are mainly utilized for colorimetric detection. Nevertheless, electrochemical paper based biosensors have been given the prime attention due to its potential of providing, low cost, and precise estimation of target bio-analytes. In addition to this, such electrochemical prototypes have the potentials of being integrated with the electronic data requisition systems. The modification of insulator type paper was done by doping with conducting polymers and nano-composites, making them a semiconductor or conductor (Narang et al., 2017; Safavieh et al., 2017), which allows their use in electrochemical sensors. In this context a reduced graphene was doped with the paper material to form the reduced graphene paper (Kumar et al., 2015b). Here, the excellent conducting property of graphene has been exploited to make the paper matrix conducting which was utilized for the detection of CEA, with high sensitivity of 25.8 μA ng^{-1} mL cm^{-2} (Kumar et al., 2015b). Similarly, conducting nano-conjugated system has also been explored to make the conducting paper suitable for high performance biosensing mechanism with improved analytical capacities (Ruecha et al., 2014). Apart

from these, interdisciplinary research practices have introduced the hybrid paper based microfluidic channels. In general, such channels are fabricated by using various polymeric counterparts such as PDMS, poly vinyl alcohol, and several other hydrophobic polymers, which has also been introduced with the hydrophobic ink. For instance, in a report published by Nie *et al.* a paper based biosensing platform using permanent board marker plotting PSA have been reported with the excellent DL and LR of 360.2 ng $L^{-1}$and 0.5-50 μg L^{-1}, respectively (Nie et al., 2012). In another report, Zuo *et al.*, developed PDMS/paper/glass hybrid microfluidic system for *Lactobacillus acidophilus* detection with DL of 11.0 cfu mL^{-1} (Zuo et al., 2013).

3.1.2. Improvement based on design optimized strategies

Design or patterned structures are evolutionary optimized adaption of nature, it optimizes different forces acting on any object and thus delivers the controlled fundamental physical phenomenon (Prasad et al., 2016). Designing in micro-scales explores various unique properties of matters. For example, capillary filling properties of paper enhance the fluids flow in micro-channels in hybridized microfluidic devices. In an article reported by Arun *et al.* on such microfluidic based fluid handling prototypes where the paper-PDMS hybrid channels show enhanced fluid flow compared to only PDMS based channel (Arun et al., 2016). In paper based platforms, the analytical performance is substantially improved by directing the fluid flow by incorporating guiding barriers and addition of pre-processing units. In an attempt, Fridley *et al.* have reported 3.0 fold signal amplifications by employing consistent controlled rehydration of reagents. In addition to this, when this prototype was customized for the malarial detection, an improved DL of 3.6 ng/ml which was attained (Fridley et al., 2014). Similarly, Fu *et al.* have reported an extended LFA based on functionalized nanoparticle with multistep chemical processing which shows 4.0 fold amplified signals (Fu et al., 2012). Apart from these examples, there are several other advancements which have been reported including the "three dimensional pop up" based glucometer coupled electrochemical biosensors for detection of beta-hydroxybutyrate with DL of 0.3 mM (Wang et al., 2016). Moreover, paper based designs constantly met the interventions of technological excellences in various prototypes from origami to Z-folded simplistic designs to supply the demand of affordable, efficient, and ASSURED diagnostic devices. For instance, Z-folded biosensing device have been developed by Jin and coworkers for ATP quantification based of *Salmonella* detection with DL of 2.6×10^{7} CFU mL^{-1} (Jin et al., 2015). In an origami based prototype reported by Liu and Crooks, an efficient use of designing in paper based microfluidic devices has been elegantly shown (Liu and Crooks, 2011). Depending on the sample's physical conditions and compositions, a preprocessing step is required to screen out the bulk and interfering moieties. Thus, the preprocessing of bio-fluid before feeding into

diagnostic devices plays a crucial role in obtaining results. As in case of blood sample, without being preprocessed, diagnostic result may lead to false positive or negative output which can be avoided by using paper based absorbing pad to filter out the flow of hindering molecules from sample. For instance, a paper based microfluidic device was designed with such preprocessing unit that has been utilized for liver functioning test by detecting alkaline phosphatase, aspartate aminotransferase, and total serum protein with the DL of 44 U L^{-1}, 15 U L^{-1}, and 7 g L^{-1}, respectively (Vella et al., 2012) . Furthermore, technological advancements exploring unique behaviors of nanomaterials / hybrid systems/ bioconjugates has significantly improved the paper based diagnostics (Chandra, 2016). Various sensing mechanism, target biomarkers, analytical performance, and sample type of paper based biosensor has been summarized in Table 2.

3.2. Improving the signal by refining biosensing mechanisms

Biosensing mechanisms can also compensate the limitations of matrix bourn noises by increasing the signal output or by signal amplification strategies. These mechanisms directly influence analytical performance, which are determining measures for the quality of health monitoring biosensors (Chandra, 2016). Unfortunately, in current scenarios, paper based platforms are lagging behind due to various limitations. Thus, several strategies have also been adopted to improvise such shortcomings by using either enzyme or enzyme mimetic systems**.**

3.2.1. Enzyme assisted strategies

In biosensing applications, enzymes are predominately used as bio-recognition elements due to the excellent selectivity. Usually, colorimetric detections follow the release of electrons with the help of an enzyme which subsequently alters the oxidation state of chromophoric agent present in it. So far, various analytes have been detected using colorimetric detections such as; antibodies (Cheng et al., 2010), bacteria (Jokerst et al., 2012), cells (Jin et al., 2015), heavy metals (Hossain and Brennan, 2011; Nath et al., 2014, 2015), and several clinically important molecules (Cha et al., 2012; Chen et al., 2012; Klasner et al., 2010; Vella et al., 2012; W et al., 2010). Apart from these, enzymes have also been used in paper based electrochemical biosensors to detect several biomarkers. For instance, Zang *et al.* have proposed an origami based 3D paper electro-immunosensor using HRP mediated enzymatic systems (HRP-O-phenylenediamine-H_2O_2) for the detection of clinically important molecules (Zang et al., 2012), where the device has been utilized to assess the different cancer biomarkers including carcinoma antigen 125 (CA125), carcinoma antigen 199 (CA199), alpha-fetoprotein (AFP), and CEA as model targets and reported the DL of 6.0 mU mL^{-1}, 8.0 mU mL^{-1}, 0.01ng mL^{-1}, and 5.0 pg mL^{-1}, respectively. Using other strategies the electrochemical paper based immunodevices have been reported for the cancerous

biomarkers such as; CA125 and carbohydrate antigen 153 (CA153) with DL of 0.05 ng mL^{-1} and 0.05 ng mL^{-1}, respectively (Wu et al., 2014). Additionally, in this investigation the biosensing signals have been amplified with the help of graphene composites. Furthermore, an electrochemical biosensing prototype proposed by Li *et al.* to detect PSA using the GOx as and redox initiator, and 3,3',5,5'-Tetramethylbenzidine (TMB) as chromophoric agent which shows high sensitivity, specificity, and excellent performance in human serum sample with LR between 0.005 to 100 ng mL^{-1} and DL of 0.0012 ng mL^{-1} (Li et al., 2014). Apart from these, various clinically important molecules such as; acetoacetate, ketones, glucose, and nitrites have also been detected in bio-fluids using paper biosensors, utilizing enzyme assisted strategies (Cha et al., 2012; Chen et al., 2012; Klasner et al., 2010).

3.2.2. Nanomaterial assisted strategies

The biggest limitations associated with enzymes are thermal stability and lesser shelf life. Since, longer shelf life is an important parameter of commercially success; therefore enzymes are constantly being replaced by enzyme mimetic systems. Nanoparticles, owing to its excellent catalytic properties and charge carrying abilities have been widely used for biosensing mechanisms withdrawing the dependencies of enzymes (Chandra et al., 2013; Fridley et al., 2014). Nanoparticle based strategies are advantageous over the enzyme based assays in majorly two contexts. First, the nanoparticles provide simpler and straight forward methods known for the immobilizing the bio-recognition elements. Secondly, they also show robust catalytic effects to many of substrates forming enzyme mimetic systems (Taton et al., 2000) . In one work, silver nanoparticle has been used for the enzyme mimetic electrochemical paper based biosensors showing excellent sensitivity attaining DL of 767 fM (Scida et al., 2014).

3.2.3. Aptamer based strategies

Aptamers are the single stranded DNA or RNA molecules showing high affinity and specificity toward the preselected target molecules such as peptide, proteins, and various small molecules. Owing the temperature tolerance properties and high specificity, aptamers are getting prime importance in various enzyme mimetic or replacement strategies (Chandra et al., 2011). Aptamers are also used as a biosensing recognition element in different formats of paper biosensors using optical, electrochemical readout modes. For instance, a paper based fluorescence aptasensor has been developed by Liang *et al.* for multiplex cancer cell detection showing DL of 62, 70, and 65 cells mL^{-1} with LR from 180 to 8×10^7, 210 to 7×10^7, and 200 to 7×10^7 cells mL^{-1} for MCF-7, HL-60, and K562 cells, respectively (Liang et al., 2016). In addition to this, Zhang *et al.* have also designed an aptamer based μPAD for adenosine detection which is a cofactor for various biologically important functions, attaining the DL of 0.16 μM

(Zhang et al., 2016). The most advance paper based origami electrochemical microfluidics device has been developed for adenosine detection with DL of 11.8 μM (Liu et al., 2012b). Similarly, a paper based microfluidic device has been developed using aptamer as biorecognition element for peptide. The DL and LR have been reported with 8.33 pM and 25 pM to 50 nM, respectively (Ma et al., 2017).

4. Conclusions and future perspective

Although, the concept of POCT devices initially originated to serve various diagnoses of diseases, but down the age's paradigm have been shifted towards their utility for achieving the state of wellness. Tremendous researches have been done to improvise the POCT device to make available for all groups in society. Recently, paper based technologies have been found great attentions due to its capabilities of serving the demands of various end-users and manufacturer. So far several works have been done on paper based microfluidic devices leaving no stone unturned. In addition to this, tremendous efforts have also been taken to achieve the low cost sensitive detections. Researchers have also reported multiplexing paper based POCT devices in order to further reduce of cost burden to the end users. Unfortunately, in spite of rigorous research and development activities on paper based biosensors, results provided are majorly qualitative or semi-quantitative, therefore still require improvements. These can be resolved by adopting various advance engineering and manufacturing processes. For commercial success of these POCT devices, investigation of paper type and introduction of state-of-the-art technologies for stable mass manufacturing is important and encouraging. It is anticipated that more detail and extensive study of paper based biosensors will paved way for their application in personalized diagnosis, which may eventually decrease the disease burden and increase quality of human life.

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References

Academy, P., 2016. Fibrous Materials for Paper. Paper Academy.
Agrawal, B., Chandra, P., Goyal, R.N., Shim, Y.-B., 2013. Biosens. Bioelectron. 47, 307-312.
Ahmed, S., Bui, M.-P.N., Abbas, A., 2016. Biosens. Bioelectron. 77, 249-263.
Apilux, A., Ukita, Y., Chikae, M., Chailapakul, O., Takamura, Y., 2013. Lab Chip 13, 126-135.
Arun, R.K., Priyadarshini, N., Chaudhury, K., Chanda, N., Biswas, G., Chakraborty, S., 2016. J. Micromech. Microeng. 26, 105008.
Bahadır, E.B., Sezgintürk, M.K., 2016. TrAC Trends in Anal. Chem. 82, 286-306.
Bodelón, G., Mourdikoudis, S., Yate, L., Pastoriza-Santos, I., Pérez-Juste, J., Liz-Marzán, L.M., 2014. ACS Nano 8, 6221-6231.
Boonyasit, Y., Chailapakul, O., Laiwattanapaisal, W., 2016. Anal. Chim. Acta 936, 1-11.
Britannica, O.E., 2016. Papermaking
Butts, C., Kamel–Reid, S., Batist, G., Chia, S., Blanke, C., Moore, M., Sawyer, M.B., Desjardins, C., Dubois, A., Pun, J., Bonter, K., Ashbury, F.D., 2013. Current Oncology 20, e475-e483.
Cao, R., Tian, W., Shen, W., 2017. Talanta 165, 357-363.
Carrilho, E., Martinez, A.W., Whitesides, G.M., 2009. Anal. Chem. 81, 7091-7095.
Carvalhal, R.F., Simão Kfouri, M., de Oliveira Piazetta, M.H., Gobbi, A.L., Kubota, L.T., 2010. Anal. Chem. 82, 1162-1165.
Cha, R., Wang, D., He, Z., Ni, Y., 2012. Carbohydr. Polym. 88, 1414-1419.
Chandra, P., 2016. Nanobiosensors for Personalized and Onsite Biomedical Diagnosis. IET, London.
Chandra, P., Das, D., Abdelwahab, A.A., 2010. Dig. J. Nanomater. Biostruct.5.
Chandra, P., Koh, W.C.A., Noh, H.-B., Shim, Y.-B., 2012. Biosens. Bioelectron. 32, 278-282.
Chandra, P., Noh, H.-B., Pallela, R., Shim, Y.-B., 2015. Biosens.Bioelectron. 70, 418-425.
Chandra, P., Noh, H.-B., Won, M.-S., Shim, Y.-B., 2011. Biosens. Bioelectron. 26, 4442-4449.
Chandra, P., Singh, J., Singh, A., Srivastava, A., Goyal, R.N., Shim, Y.B., 2013. J Nanopart. Article ID 535901, 1-12.
Chen, X., Chen, J., Wang, F., Xiang, X., Luo, M., Ji, X., He, Z., 2012. Biosens. Bioelectron. 35, 363-368.
Cheng, C.-M., Martinez, A.W., Gong, J., Mace, C.R., Phillips, S.T., Carrilho, E., Mirica, K.A., Whitesides, G.M., 2010. Angew. Chem. Int. Ed. (English) 49, 4771-4774.
Chin, C.D., Linder, V., Sia, S.K., 2012. Lab Chip 12, 2118-2134.
Choi, J.R., Hu, J., Tang, R., Gong, Y., Feng, S., Ren, H., Wen, T., Li, X., Wan Abas, W.A.B., Pingguan-Murphy, B., Xu, F., 2016. Lab Chip 16, 611-621.
Choudhary, M., Yadav, P., Singh, A., Kaur, S., Ramirez-Vick, J., Chandra, P., Arora, K., Singh, S.P., 2016. Electroanalysis.
Chun, H.J., Park, Y.M., Han, Y.D., Jang, Y.H., Yoon, H.C., 2014. BioChip J. 8, 218-226.
Cinti, S., Basso, M., Moscone, D., Arduini, F., 2017a. Anal. Chim. Acta. 960, 123-130.
Cinti, S., Minotti, C., Moscone, D., Palleschi, G., Arduini, F., 2017b. Biosens. Bioelectron. 93, 46-51.
Credou, J., Berthelot, T., 2014. J. Mater. Chem. B 2, 4767-4788.
Dai, G., Hu, J., Zhao, X., Wang, P., 2017. Sens. Actuators B: Chem. 238, 138-144.
Damborsky, P., Koczula, K.M., Gallotta, A., Katrlik, J., 2016. Analyst 141, 6444-6448.
Delaney, J.L., Hogan, C.F., Tian, J., Shen, W., 2011. Anal. Chem. 83, 1300-1306.
Dias, A.A., Cardoso, T.M.G., Cardoso, R.M., Duarte, L.C., Muñoz, R.A.A., Richter, E.M., Coltro, W.K.T., 2016. Sens. Actuators B: Chem. 226, 196-203.
Doty, A., Gaskell, S., Sultan, F., 2012. Assey System Components. IVD Technology, p. 5.
Dungchai, W., Chailapakul, O., Henry, C.S., 2009. Anal. Chem. 81, 5821-5826.
Dungchai, W., Chailapakul, O., Henry, C.S., 2011. Analyst 136, 77-82.
Ellerbee, A.K., Phillips, S.T., Siegel, A.C., Mirica, K.A., Martinez, A.W., Striehl, P., Jain, N., Prentiss, M., Whitesides, G.M., 2009. Anal. Chem. 81, 8447-8452.
Fischer, C., Fraiwan, A., Choi, S., 2016. Biosens. Bioelectron. 79, 193-197.

Free, H.M., Collins, G.F., Free, A.H., 1960. Clin. Chem. 6, 352-361.
Fridley, G.E., Le, H., Yager, P., 2014. Anal. Chem. 86, 6447-6453.
Fu, E., Liang, T., Spicar-Mihalic, P., Houghtaling, J., Ramachandran, S., Yager, P., 2012. Anal. Chem. 84, 4574-4579.
Ge, L., Yu, J., Ge, S., Yan, M., 2014. Analytical and Bioanal. Chem. 406, 5613-5630.
Geffroy, C., Labeau, M.P., Wong, K., Cabane, B., Cohen Stuart, M.A., 2000. Colloids Surf., A 172, 47-56.
Gubala, V., Harris, L.F., Ricco, A.J., Tan, M.X., Williams, D.E., 2012. Anal. Chem. 84, 487-515.
Hood, L., 2013. Rambam Maimonides Med. J. 4, e0012.
Hossain, S.M.Z., Brennan, J.D., 2011. Anal. Chem. 83, 8772-8778.
Ibrahim, M.M., Koschella, A., Kadry, G., Heinze, T., 2013. Carbohydr. Polym. 95, 414-420.
InnovaBiosciences, 2016. Lateral Flow Immunoassays.
Isaad, J., El Achari, A., 2011. Tetrahedron Lett. 67, 4939-4947.
Jin, S.-Q., Guo, S.-M., Zuo, P., Ye, B.-C., 2015. Biosens. Bioelectron. 63, 379-383.
Jokerst, J.C., Adkins, J.A., Bisha, B., Mentele, M.M., Goodridge, L.D., Henry, C.S., 2012. Anal. Chem. 84, 2900-2907.
Karra-Châabouni, M., Bouaziz, I., Boufi, S., Botelho do Rego, A.M., Gargouri, Y., 2008. Colloids Surf., B 66, 168-177.
Kashish, Gupta, S., Dubey, S.K., Prakash, R., 2015. Anal. Methods 7, 2616-2622.
Kennedy, J.F., Quinton, L.A., 2000. Carbohydr. Polym. 43, 206-207
Klasner, S.A., Price, A.K., Hoeman, K.W., Wilson, R.S., Bell, K.J., Culbertson, C.T., 2010. Anal. Bioanal. Chem. 397, 1821-1829.
Kumar, A., Hens, A., Arun, R.K., Chatterjee, M., Mahato, K., Layek, K., Chanda, N., 2015a. Analyst 140, 1817-1821.
Kumar, S., Kumar, S., Srivastava, S., Yadav, B.K., Lee, S.H., Sharma, J.G., Doval, D.C., Malhotra, B.D., 2015b. Biosens. Bioelectron. 73, 114-122.
Lei, K.F., Yang, S.-I., Tsai, S.-W., Hsu, H.-T., 2015. Talanta 134, 264-270.
Li, C.-z., Vandenberg, K., Prabhulkar, S., Zhu, X., Schneper, L., Methee, K., Rosser, C.J., Almeide, E., 2011. Biosens. Bioelectron. 26, 4342-4348.
Li, L., Xu, J., Zheng, X., Ma, C., Song, X., Ge, S., Yu, J., Yan, M., 2014. Biosens. Bioelectron. 61, 76-82.
Li, M., Tian, J., Al-Tamimi, M., Shen, W., 2012a. Angew. Chem. Int. Ed. (English) 51, 5497-5501.
Li, X., Ballerini, D.R., Shen, W., 2012b. Biomicrofluidics 6, 011301.
Liang, L., Su, M., Li, L., Lan, F., Yang, G., Ge, S., Yu, J., Song, X., 2016b. Sens. Actuators B: Chem. 229, 347-354.
Liu, F., Zhang, C., 2015. Sens. Actuators B: Chem. 209, 399-406.
Liu, H., Crooks, R.M., 2011. J. Am. Chem. Soc. 133, 17564-17566.
Liu, H., Xiang, Y., Lu, Y., Crooks, R.M., 2012a. Angew. Chem. Int. Ed. (English) 51, 6925-6928.
Liu, H., Xiang, Y., Lu, Y., Crooks, R.M., 2012b. Angew. Chem. 124, 7031-7034.
Liu, L., Peng, C., Jin, Z., Xu, C., 2007. Biomed. Chromatogr. 21, 861-866.
Liu, X., Mwangi, M., Li, X., O'Brien, M., Whitesides, G.M., 2011. Lab Chip 11, 2189-2196.
Ma, C., Liu, H., Zhang, L., Li, L., Yan, M., Yu, J., Song, X., 2017. ChemElectroChem.
Ma, X., Chen, Z., Kannan, P., Lin, Z., Qiu, B., Guo, L., 2016. Anal. Chem. 88, 3227-3234.
Martinez, A.W., Phillips, S.T., Butte, M.J., Whitesides, G.M., 2007. Angew. Chem. Int. Ed. (English) 46, 1318-1320.
Martinez, A.W., Phillips, S.T., Carrilho, E., Thomas, S.W., Sindi, H., Whitesides, G.M., 2008a. Anal. Chem. 80, 3699-3707.
Martinez, A.W., Phillips, S.T., Wiley, B.J., Gupta, M., Whitesides, G.M., 2008b. Lab Chip 8, 2146-2150.
Maurya, P.K., Kumar, P., Chandra, P., 2016. Arch. Physiol. Biochem. 122, 61-66.
Millipore, M., 2013. Rapid Lateral Flow Test Strips: Considerations for Product Development. In: Millipore, M. (Ed.). Merck Millipore, Billerica, MA.

Narang, J., Malhotra, N., Singhal, C., Mathur, A., Chakraborty, D., Anil, A., Ingle, A., Pundir, C.S., 2017. Biosens. Bioelectron. 88, 249-257.
Nath, P., Arun, R.K., Chanda, N., 2014. RSC Adv. 4, 59558-59561.
Nath, P., Arun, R.K., Chanda, N., 2015. RSC Adv. 5, 69024-69031.
Nery, E.W., Kubota, L.T., 2016. J. Pharm. Biomed. Anal. 117, 551-559.
Nie, J., Zhang, Y., Lin, L., Zhou, C., Li, S., Zhang, L., Li, J., 2012. Anal Chem. 84, 6331-6335.
Nie, Z., Nijhuis, C.A., Gong, J., Chen, X., Kumachev, A., Martinez, A.W., Narovlyansky, M., Whitesides, G.M., 2010. Lab Chip 10, 477-483.
Noh, H.-B., Chandra, P., Moon, J.O., Shim, Y.-B., 2012. Biomaterials 33, 2600-2607.
Nurul Najian, A.B., Engku Nur Syafirah, E.A.R., Ismail, N., Mohamed, M., Yean, C.Y., 2016. Anal. Chim. Acta 903, 142-148.
Obama, B., 2016. JAMA 316, 525-532.
Oblath, E.A., Henley, W.H., Alarie, J.P., Ramsey, J.M., 2013. Lab Chip 13, 1325-1332.
Pallela, R., Chandra, P., Noh, H.-B., Shim, Y.-B., 2016. Biosens. Bioelectron 85, 883-890.
Parrilla, M., Cánovas, R., Andrade, F.J., 2017. Biosens. Bioelectron. 90, 110-116.
Posthuma-Trumpie, G.A., Korf, J., van Amerongen, A., 2009. Anal. Bioanal. Chem. 393, 569-582.
Prasad, A., Mahato, K., Chandra, P., Srivastava, A., Joshi, S.N., Maurya, P.K., 2016. Journal of Molecular and Engineering Materials 4, 1640004.
Rassaei, L., Olthuis, W., Tsujimura, S., Sudhölter, E.J.R., van den Berg, A., 2014. Anal. Bioanal. Chem. 406, 123-137.
Rezk, A.R., Qi, A., Friend, J.R., Li, W.H., Yeo, L.Y., 2012. Lab Chip 12, 773-779.
Roberts, J.C., 1996. The paper formation process. In: Roberts, J.C. (Ed.), The Chemistry of Paper, pp. 69-88. The Royal Society of Chemistry.
Rohrman, B.A., Leautaud, V., Molyneux, E., Richards-Kortum, R.R., 2012. PLoS ONE 7, e45611.
Ruecha, N., Rangkupan, R., Rodthongkum, N., Chailapakul, O., 2014. Biosens. Bioelectron. 52, 13-19.
Safavieh, M., Kaul, V., Khetani, S., Singh, A., Dhingra, K., Kanakasabapathy, M.K., Draz, M.S., Memic, A., Kuritzkes, D.R., Shafiee, H., 2017. Nanoscale 9, 1852-1861.
Scida, K., Cunningham, J.C., Renault, C., Richards, I., Crooks, R.M., 2014. Anal. Chem. 86, 6501-6507.
Scotton, C., Passarelli, C., Neri, M., Ferlini, A., 2014. Exp. Cell. Res. 325, 44-49.
Shafiee, H., Asghar, W., Inci, F., Yuksekkaya, M., Jahangir, M., Zhang, M.H., Durmus, N.G., Gurkan, U.A., Kuritzkes, D.R., Demirci, U., 2015. Sci. Rep. 5, 8719
Shah, A., 2016. global Health Overview.
Shi, C.-G., Shan, X., Pan, Z.-Q., Xu, J.-J., Lu, C., Bao, N., Gu, H.-Y., 2012. Anal. Chem. 84, 3033-3038.
Singh, P., Nath, P., Arun, R.K., Mandal, S., Chanda, N., 2016. RSC Adv. 6, 92729-92738.
Snyderman, R., 2012. Biotechnology Journal 7, 973-979.
Songjaroen, T., Dungchai, W., Chailapakul, O., Henry, C.S., Laiwattanapaisal, W., 2012. Lab Chip 12, 3392-3398.
Soni, A., Jha, S.K., 2015. Biosens. Bioelectron. 67, 763-768.
Struss, A., Pasini, P., Ensor, C.M., Raut, N., Daunert, S., 2010. Anal. Chem. 82, 4457-4463.
Sun, G., Wang, P., Ge, S., Ge, L., Yu, J., Yan, M., 2014. Biosens. Bioelectron. 56, 97-103.
Sun, Y., He, K., Zhang, Z., Zhou, A., Duan, H., 2015. Biosens. Bioelectron. 68, 358-364.
Taton, T.A., Mirkin, C.A., Letsinger, R.L., 2000. Science 289, 1757-1760.
Teengam, P., Siangproh, W., Tuantranont, A., Henry, C.S., Vilaivan, T., Chailapakul, O., 2017. Analytica Chimica Acta 952, 32-40.
Thakur, B., Jayakumar, S., Sawant, S.N., 2015. Chem. Commun. 51, 7015-7018.
The Dow Chemical Company, 2016. Nitrocellulose. Link: http://www.dow.com
Vashist, S.K., Venkatesh, A.G., Zengerle, R., Stetten, F.v., Luong, J.H., 2016. Smartphone-based *in vitro* diagnostic technologies for personalized healthcare monitoring and management. Nanobiosensors for Personalized and Onsite Biomedical Diagnosis, pp. 231-251. IET, London.
Vella, S.J., Beattie, P., Cademartiri, R., Laromaine, A., Martinez, A.W., Phillips, S.T., Mirica, K.A., Whitesides, G.M., 2012. Anal. Chem. 84, 2883-2891.

Dungchai,W., Chailapakul, O., Henry C. S., 2010. Anal. Chim. Acta 674, 227.
Wang, C.-C., Hennek, J.W., Ainla, A., Kumar, A.A., Lan, W.-J., Im, J., Smith, B.S., Zhao, M., Whitesides, G.M., 2016. Anal. Chem. 88, 6326-6333.
Wang, J., Bowie, D., Zhang, X., Filipe, C., Pelton, R., Brennan, J.D., 2014. Chem. Mater. 26, 1941-1947.
Wang, P., Ge, L., Yan, M., Song, X., Ge, S., Yu, J., 2012a. Biosens. Bioelectron. 32, 238-243.
Wang, S., Ge, L., Song, X., Yu, J., Ge, S., Huang, J., Zeng, F., 2012b. Biosens. Bioelectron. 31, 212-218.
Wang, X., Gartia, M.R., Jiang, J., Chang, T.-W., Qian, J., Liu, Y., Liu, X., Liu, G.L., 2015. Sens. Actuators B: Chem. 209, 677-685.
Wang, Y., Wang, S., Ge, S., Wang, S., Yan, M., Zang, D., Yu, J., 2013. Anal. Methods 5, 1328-1336.
Websource, 2016. http://www.grandviewresearch.com/press-release/global-paper-diagnostics-market p. 108. Report ID: 978-1-68038-725-4.
Wen, J., Chen, J., Zhuang, L., Zhou, S., 2016. Biosens.Bioelectron. 79, 656-660.
Won, S.-Y., Chandra, P., Hee, T.S., Shim, Y.-B., 2013. Biosens. Bioelectron. 39, 204-209.
Wu, L., Ma, C., Zheng, X., Liu, H., Yu, J., 2015. Biosens. Bioelectron. 68, 413-420.
Wu, Y., Xue, P., Hui, K.M., Kang, Y., 2014. Biosens. Bioelectron. 52, 180-187.
Wu, Y., Xue, P., Kang, Y., Hui, K.M., 2013. Anal. Chem. 85, 8661-8668.
Xia, Y., Si, J., Li, Z., 2016. Biosens. Bioelectron. 77, 774-789.
Yetisen, A.K., Akram, M.S., Lowe, C.R., 2013. Lab Chip 13, 2210-2251.
Yildiz, U.H., Alagappan, P., Liedberg, B., 2013. Anal. Chem. 85, 820-824.
Yin, H., Wang, M., Li, B., Yang, Z., Zhou, Y., Ai, S., 2015. Biosens. Bioelectron. 63, 26-32.
You, D.J., Park, T.S., Yoon, J.-Y., 2013. Biosens. Bioelectron. 40, 180-185.
Yu, J., Ge, L., Huang, J., Wang, S., Ge, S., 2011a. Lab Chip 11, 1286-1291.
Yu, J., Wang, S., Ge, L., Ge, S., 2011b. Biosens. Bioelectron. 26, 3284-3289.
Yu, T., Dai, P.-P., Xu, J.-J., Chen, H.-Y., 2016. ACS Appl. Mater. Interfaces 8, 4434-4441
Zang, D., Ge, L., Yan, M., Song, X., Yu, J., 2012. Chem. Commun. 48, 4683-4685.
Zhang, Y., Gao, D., Fan, J., Nie, J., Le, S., Zhu, W., Yang, J., Li, J., 2016. Biosens. Bioelectron 78, 538-546.
Zhao, C., Thuo, M.M., Liu, X., 2013. Sci. Tech. Adv. Mater. 14, 054402.
Zhao, W., Ali, M.M., Aguirre, S.D., Brook, M.A., Li, Y., 2008. Anal. Chem. 80, 8431-8437.
Zhong, Z.W., Wang, Z.P., Huang, G.X.D., 2012. Microsyst. Technol. 18, 649-659.
Zhu, Y., Chandra, P., Shim, Y.-B., 2012a. Anal. chem. 85, 1058-1064.
Zhu, Y., Chandra, P., Song, K.-M., Ban, C., Shim, Y.-B., 2012b. Biosens. Bioelectron. 36, 29-34.
Zhybak, M., Beni, V., Vagin, M.Y., Dempsey, E., Turner, A.P.F., Korpan, Y., 2016. Biosen. Bioelectron. 77, 505-511.
Zuo, P., Li, X., Dominguez, D.C., Ye, B.-C., 2013. Lab Chip 13, 3921-3928.

Figure 1: Pictorial representation of personalized health care showing its various elements and comparison between conventional and paper based device characteristics.

Figure 2: Commercially available bench top POCT devices. (a) VetScan2 (http://www.abaxis.com/);copyright © 2016, Abaxis Global diagnostics (b) ePOC® reader and host; (c) Abbott iSTAT® (www.abbottpointofcare.com); copyright © 2016 Abbott; (d) Claros® blood analyzer (http://www.eclipsepd.com/), copyright © 2016; eclipsepd; (e) Cepheid's portable DNA-reader(http://www.cepheid.com/), copyright © 2016 Cepheid; (f) Alere® triage meter pro analyzer (www.alere.com); copyright © 2016 Alere; and paper based colorimetric lateral flow diagnostic strips (g); (h); (i); (j); (k); (l); (m); (n) adopted from dipstick type LFA based colorimetric biosensors, reprinted with permission from Yetisen et al. Copyright (2013) The Royal Society of Chemistry

Figure 3: Sensing mechanism of various paper based biosensors: (a) reaction mechanism of colorimetric dipstick for the uric acid detection, adopted from Kumar *et al*. Copyright (2015) The Royal Society of Chemistry; (b) basic flow mechanism of LFA prototype, reprinted with permission from Damborsky *et al*. Copyright (2016) The Royal Society of Chemistry; (c) smartphone assisted paper based biosensor used for bacteria detection, reprinted with the permission from Shafiee *et al*. (2015)copyright Nature Publishing Group; (d) µ-PAD based paper diagnostics coupled with dedicated analyzer for beta-hydroxybutyrate detection, reprinted with permission from Wang *et al*. Copyright (2016) The American Chemical Society

Figure 4: SWOT analysis of paper based biosensors

Table 1: Different types of the paper based biosensors with advantages and disadvantages

GENRES OF PAPER BASED BIOSENSOR	POSSIBLE DETECTION MODE	ADVANTAGES	LIMITATIONS	COMMERCIALLY AVAILABLE VOLUMES
Dipstick	• Optical	• Simple design • Rapid standardization	• Supports one step process • Optical mode of detection, no variation in readout is possible • Preferably qualitative	• Urisys 11001 • Urine Analyser (Roche). • Multistix110 • Medi-Test Combi 11 • Chemstrip1 Micral test strips • Multistix 110SG reagent strips

LFA	• Optical • Electrochemical	• Adaptable sample movement • Electrochemical recognition mode possible • quantification	• Extended optimization process, and time consuming fabrication procedure • Reasonably low volume handling which require trained personals	• Sofia fluorescent immunoassay analyser • BD Veritor™ system • SNAP1 reader • Astute140™
µPAD	• Optical • Electrochemical • Chemi-luminescence • MEMS	• Provides flexible flow of sample • Available diversified methods for quantitative detections	• Ultra-low sample volume (less than 10 mL) • Doesn't Support mass production in the large volumes • Takes long optimization time and require experts	• No commercial volumes available (possibly, because of manufacturing limitations in large scale)

Table 2. Paper-based biosensor prototypes for health monitoring (from 2007 to 2017) (NR: not reported)

MODEL SPECIFICATION/ FABRICATION TECHNIQUE	SENSING MECHANISM	TARGET MOLECULE	ANALYTICAL PERFORMENCE	REAL SAMPLE	REFERENCE
Multiplex dipstick was fabricated	Colorimetric mode of detection based on enzymatic	Glucose and bovine	DL: 2.5 mM (glucose)	Urine	(Martinez et al., 2007)

using photoresist assisted patterning **Technique used :** Hydrophobic Sealing	oxidation of iodide which occurs in presence of glucose. Conversion of sample from clear to brown In BSA assay the colour of terabromophenol turns in to yellow from blue to provide analytical signal	serum albumin (BSA)	0.38 µM (BSA)		
Colorimetric sensor for endonuclease detection **Technique used:** AuNP deposition	Colorimetric signal was obtained from the cleavage of DNA. Color changes from blue to red	Endonuclease (DNase I)	NR	NR	(Zhao et al., 2008)

Electrochemical screen printed biosensor **Techniques used:** Photolithography, Screen printing	The signal was obtained using HRP- H_2O_2 with "Prussian Blue" redox mediator where presence of glucose elicit the electrochemical signal after being catalyzed	Glucose	DL: 4.9 ± 0.6 mM (Glucose)	Serum	(Dungchai et al., 2009)
Dipstick type colorimetric biosensor for bacterial quorum sensing **Technique used:** X-gal operon positive *E.*	Test strip was constructed by depositing inducible-operon-active whole-cells for X-gal where the presence of N-acylhomoserine lactone facilitate the X-gal decompose	N-acylhomoserine lactones	DL: 1 × 10^{-8} M	Saliva	(Struss et al., 2010)

coli deposition on the paper strips	facilitating the colorimetric signal				
µPAD for chemiluminescence biosensor **Techniques used:** Manual and automated X-Y plotter knives	The H_2O_2 evolution from catalysis of target molecule due to oxidase activity of enzymes (glucose and urate oxidase). The chemiluminescent property appears when H_2O_2 comes in the contact of rhodamine derivative molecules	Glucose Uric acid	DL: 0.14 mmol L^{-1} (Glucose) 0.52 mmol L^{-1} (Uric acid)	Mimicked urine	(Yu et al., 2011)
Electro-chemiluminescent detection	Electro-chemilumiescent signal was	DBAE and NADH	DL: 0.9 µM (DBAE)	NR	(Delaney et al., 2011)

of DBAE and NADH **Techniques used:** Inkjet printing and screen-printing	obtained by the reaction of tris (2,2′ - bipyridyl)ruthenium (II) ($Ru(bpy)_3^{2+}$) with target analytes		72 µM (NADH)		
Multiplex testing disc **Technique used:** Nanoparticle Deposition	The colorimetric signal was obtained when antibody functionalized AuNPs reacted with analytes in LFA due to the aggregation of nanoparticles in the detection band	*Pseudomonas aeruginosa,* *Staphylococcus aureus*	DL: 100 CFU (for both)	Physiological fluid	(Li et al., 2011)
3D µPAD for bi-enzymatic	Signal obtained by using	Glucose and uric acid	DL: On paper	Serum	(Chen et al., 2012)

colorimetric analysis **Technique used;** Lithography	peroxidase (HRP or Urate oxidase) - H_2O_2 systems, where the presence of glucose and uric acid selectively catalyzed, eliciting the redox signal to alter the oxidation state of chromogenic substance		strip : 3.81×10^{-5} M (Glucose) 4.31×10^{-5} M (Uric acid) On camera : 2.13×10^{-4} M (Glucose) 2.87×10^{-4} M (Uric acid)		
3D electrochemical immune detection based on multi-walled carbon	Signal was obtained by using HRP-Phenylenediamine-H_2O_2 electrochemical system	Cancer markers: CA125 and CEA	LR: 0.001-75.0 UmL^{-1} (CA 125) 0.05-50.0 ng mL^{-1} (CEA)	Serum	(Wang et al., 2012)

nanotubes functionalized paper **Technology:** 3D Wax patterning			DL: 0.2 mU mL^{-1} (CA125) 0.01 ng mL^{-1} (CEA)		
Colorimetric assay **Technique used:** AKD printing	Bioassay, impregnated with antisera of ABO typing where affinity binding of antigen and antibody elicit the signal	Blood typing device (ABO and RhD)	NR	Blood	(Li et al., 2012)
Self-powered origami paper analytical device (*o*PAD) **Technique used:**	Signal was obtained by biotin labelled aptamer and adenosine coupled with GOx-H_2O_2 electrochemical	Adenosine concentration	LR: 0.5 mM to 5 mM DL: 11.8 µM	NR	(Liu et al., 2012)

Thermal lamination	sensing system				
Carbon dots assisted chemiluminescence DNA biosensor **Technique used:** Wax printed electrode	Carbon dots covalently immobilized on nano-porous gold were used for the generation of chemiluminescece signal in presence of DNA and potassium permanganate	DNA	LR: 10^{-18} -10^{-14} M DL: 8.56 × 10^{-19} M	Serum	(Wang et al., 2013)
Optical fluorescence quenching based detection of miRNA **Technique**	The analytical signal was obtained from positively charged poly (3-alkoxy-4-methylthiophene) based detection of miRNA, where the	Lung cancer associated miRNA	NR	NR	(Yildiz et al., 2013)

used: Fluorescence quenching	fluorescence signal was quenched, showing the transitions in color appearance			
Multiplexed electrochemical paper based biosensor for cancer biomarkers **Technique used:** Photoresist-patterning	Signal was obtained by HRP-O-phenylenediamine-H_2O_2 electrochemical system	Cancer biomarkers: alpha-fetoprotein (AFP), CEA, CA125, and CA153	LR: AFP, CEA, CA125, and CA153; 0.001–100 ng mL^{-1}, 0.005–100 ng mL^{-1}, 0.001–100 ng mL^{-1}, and 0.005–100 ng mL^{-1}, respectively DL: AFP, CEA,	Serum (Wu et al., 2013)

			CA125, and CA153; 0.001, 0.005, 0.001, and 0.005 ng mL^{-1}, respectively		
Polydimethylsiloxane /paper/glass hybrid multiplexed microfluidic aptasensor for pathogen detection **Technique used:** Soft lithography	Fluorescence quenching occur when the fluorescent tagged aptamer reacts with graphene oxide sheets gives the full quenching of fluorescence. However, in presence of the target organism the process does	*Lactobacillus acidophilus*	LR: 9.4 to 150.0 cfu mL^{-1} DL: 11.0 cfu mL^{-1}	NR	(Zuo et al., 2013)

	not occur due to the conformational changes in fluorescent labelled aptamers facilitate the selective detection				
Automated ELISA biosensor for human chorionic gonadotropin (hCG) detection **Technique used:** Inkjet Printing	Colorimetric signal was obtained when analyte reaches to the detection zone forming a visible sandwich immunocomplex at the detection zone	hCG	DL: 0.81 ng mL^{-1}	NR	(Apilux et al., 2013)
2D nanocomposite material based	Amperometric signal was obtained by	Cholesterol	LR: 50 µM to 10 mM	Serum	(Ruecha et al., 2014)

cholesterol sensing **Technique used:** Electro-spraying	cholesterol oxidase-H_2O_2 system with G/PVP/PANI modified electrode and graphene nanocomposite consisting of poly-vinylpyrrolidone and polyaniline		DL: 1 µM		
µPAD based colorimetric detections **Technique used:** Molecular imprinting	AuNPs based paper working electrode and polypyrrole-functionalized ZnO nanoparticles based detection of the analyte where ascorbic acid assisted as nontoxic electron supplier for capturing the	Pentachlorophenol	LR: 0.01-100 ng mL^{-1} DL: 4 pg mL^{-1}	NR	(Sun et al., 2014)

	photo generated holes for stable photocurrent signal generation				
Optical biosensor for glucose monitoring **Technique used:** Wax printing	The signal was obtained by using peroxidases (GOx and HRP)- H_2O_2 based colorimetric detection system, where the presence of analyte evolves H_2O_2 that converts the 4-aminoantipyrinean d and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethylaniline sodium salt monohydrate to	Glucose	DL: 10 mM	NR	(Chun et al., 2014)

	blue color compound				
Chemilumines cent biosensor **Technique:** Wax-screen printing	The probing zone was sequentially stacked with the coupling of various chemicals with HRP tagging. Signal was obtained by HRP-luminol-PIP chemiluminescent system	Gene detection of *Listeria monocytogen es*	LR: 1.94×10^{-1} - 1.94×10^{4} pmol L^{-1} DL: 6.3×10^{-2} pmol L^{-1}	NR	(Liu and Zhang, 2015)
Label free quantitative immunoassay demonstrated by biotin-avidin binding interaction **Technique used :** Deposition of	Signal was obtained by measuring the fluctuation of current on applying avidin treatment at functionalized zone	Biotin-avidin binding	DL : 25 ng Ml^{-1}	NR	(Lei et al., 2015)

biotin functionalized CNT using vacuum filtration					
Real time electrochemical detection of H_2O_2 using CNT hybrid paper	Signal was obtained by the electro-catalysis of H_2O_2-redox at platinum electrodes	H_2O_2	LR: 25 µM DL: 10 nM	Macrophages cell model	(Sun et al., 2015)
Technique used: Sputtering deposition					
Electrochemical biosensor for the detection of nerve agent	Carbon black / prussian blue nanocomposite was used for probe fabrication in electrochemical	Nerve agents, butyrylthiocholine,	LR: 5 -100 µgL^{-1} DL: 3 µg L^{-1}	NR	(Cinti et al., 2016, 2017)

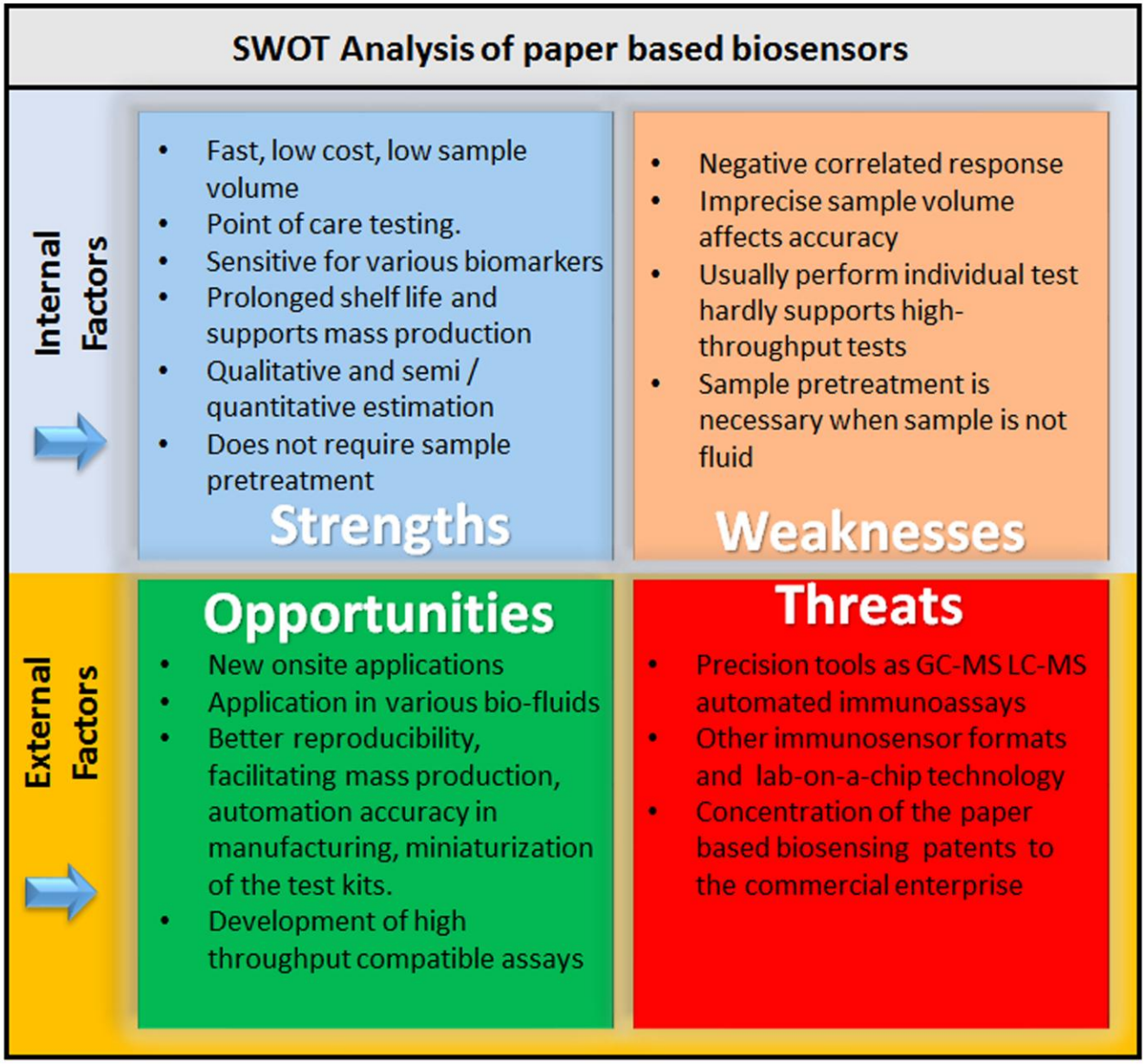
Technique used: Deposition	biosensor and the signal was obtained from enzymatic reactions catalyzed by butyrylthiocholinea sterase.			
LFA for bacterial nucleic acid detection **Techniques used:** Cell deposition	The signal was obtained from thiolated oligonucleotide (detector probe) conjugated with AuNPs when comes to the target analyte after activation	*Escherichia coli*	DL: 10 CFU mL^{-1} (PBS) 10 CFU mL^{-1} (Water) 10 CFU mL^{-1} (Milk) 10 CFU mL^{-1} (Blood) and 1000 CFU mL^{-1} (spinach)	Biological samples (Choi et al., 2016)

A 3D electrochemical glycated-hemoglobin biosensors **Techniques used :** Screen-printing Wax - patterning	Label free electrochemical sensing for the glycated hemoglobin based on the affinity binding of target proteins with "anti-glycated hemoglobin". Signal was obtained in terms of impedance change	Glycated hemoglobin	LR: Up to 20 g dL^{-1} DL: 0.08 g dL^{-1}	Blood	(Boonyasit et al., 2016)
Colorimetric sensor for lactate detection **Technique used:** Enzyme	Colorimetric responses was obtained by the formazan producing reaction using the substrate WST-8 in presence of	Lactate	LR: 0.5 - 8 mM	NR	(Dai et al., 2017)

deposition	NADH and phenazine methosulphate				
Enzyme electrode based glucose monitoring **Techniques used:** Screen printing Sputtering	The signal generation occurs by selective catalysis of glucose by GOx. The signal was obtained platinum doped paper electrode	Glucose	LR: 10^{-4} - $10^{-2.5}$ M DL: $10^{-4.5}$ M	Serum	(Parrilla et al., 2017)
Electrochemical biosensor for virus detection **Techniques used**: Inkjet Printing, screen printing	Change in impedance after biomolecular interaction	Human Papillomavirus virus	LR: 10 - 200 nM DL: 2.3 nM	DNA	(Teengam et al., 2017)

Highlights

- Paper based POCT devices for personalized healthcare, its limitations, and challenges have been discussed.
- Manufacturing technologies and strategies for increasing commercial impact of paper based biosensors have been reviewed.
- Properties and available technologies of paper biosensor have been described.
- Commercial aspects of paper biosensor have also been shown using SWOT diagram.


SWOT Analysis of paper based biosensors
Internal Factors
Fast, low cost, low sample volume
Point of care testing.
Sensitive for various biomarkers
Prolonged shelf life and supports mass production
Qualitative and semi / quantitative estimation
Does not require sample pretreatment
Strengths
Negative correlated response
Imprecise sample volume affects accuracy
Usually perform individual test hardly supports high-throughput tests
Sample pretreatment is necessary when sample is not fluid
Weaknesses
External Factors
Opportunities
New onsite applications
Application in various bio-fluids
Better reproducibility, facilitating mass production, automation accuracy in manufacturing, miniaturization of the test kits.
Development of high throughput compatible assays
Threats
Precision tools as GC-MS LC-MS automated immunoassays
Other immunosensor formats and lab-on-a-chip technology
Concentration of the paper based biosensing patents to the commercial enterprise