<Critical Reviews in Biotechnology>

Deploying aptameric sensing technology for rapid pandemic monitoring

Caleb Acquah[1,2], Michael K. Danquah[2]*, Dominic Agyei[3], Charles K.S. Moy[5], Amandeep Sidhu[1,6], Clarence M. Ongkudon[4]

[1]Curtin Sarawak Research Institute, Curtin University, Sarawak 98009, Malaysia.
[2]Department of Chemical Engineering, Curtin University, Sarawak 98009, Malaysia.
[3]Bio Engineering Laboratory, Chemical Engineering Department, Monash University, Victoria 3800, Australia
[4]Biotechnology Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, 88400, Malaysia
[5]Faculty of Engineering and Science, Curtin University, Sarawak 98009, Malaysia
[6]Faculty of Health Sciences, Curtin University, Perth 6102, Australia.

*Corresponding author's contact: mkdanquah@curtin.edu.my; +60 85 443836

ABSTRACT

The genome of virulent strains may possess the ability to mutate by means of antigenic shift and/or antigenic drift as well as being resistant to antibiotics with time. The outbreak and spread of these virulent diseases including avian influenza (H1N1), severe acute respiratory syndrome (SARS-Corona virus), cholera (*Vibrio cholera*), tuberculosis (*Mycobacterium tuberculosis*), Ebola haemorrhagic fever (Ebola Virus) and AIDS (HIV-1) necessitate an urgent attention to develop diagnostic protocols and assays for rapid detection and screening. Rapid and accurate detection of first cases with certainty will contribute significantly in preventing disease transmission and escalation to pandemic levels. As a result, there is a need to develop technologies that can meet the heavy demand of an all embedded inexpensive, specific and fast bio-sensing for the detection and screening of pathogens in active or latent forms to offer quick diagnosis and early treatments in order to avoid disease aggravation and unnecessary late treatment costs.

Nucleic acid aptamers are short, single-stranded RNA or DNA sequences that can selectively bind to specific cellular and biomolecular targets. Aptamers, as new-age bio-affinity probes, have the necessary biophysical characteristics for improved pathogen detection. This article seeks to review global pandemic situations in relation to advances in pathogen detection systems. It particularly discusses aptameric biosensing and establishes application opportunities for effective pandemic monitoring. Insights into the application of continuous polymeric supports as the synthetic base for aptamer coupling to provide the needed convective mass transport for rapid screening is also presented.

Keywords: Pandemics; Aptamers; Cell culture; Molecular Diagnostics; Bioscreening

1.0 INTRODUCTION

Pandemic is the end result of rapid infectious disease transmissions across communities and nations by pathogenic micro-organisms. The transmission of the causative pathogen is mostly vectored through the consumption and/or exchange of food, water, air and body fluids. The duration of the peak outbreak of a pandemic usually last longer than most public health emergencies (Ryan and Glarum, 2008; Thomas and Lavender, 2008). Most of the previous pandemic outbreaks occurred in waves with high morbidity and mortality cases separated by months. Although the outcome of the next pandemic is unknown, its occurrence and eventual effect are expected to be massive on economic, social and healthcare efforts (Ryan and Glarum, 2008). An in-depth epidemiological knowledge of disease causing agents and emerging disease pathogens is necessary to combat future pandemics. Various researchers have developed effective mechanisms essential to slow infection rates by interfering with the mode or medium of transmission(Gao *et al.*, 2013; Lee *et al.*, 2008; Sakurai *et al.*, 2014; Wolun-Cholewa *et al.*, 2013; Zhao *et al.*, 2008). Peterson *et al.* (2001) highlighted the significance of improved vaccination and antibiotic therapy in the prevention and treatment of diseases. Velusamy *et al.* (2010) reported on the importance of biosensors in detecting pathogenic microbes present in food substances as a fundamental approach to prevent diseases in humans. Pike *et al.* (2010) also discussed the horizontal transmission of pathogenic micro-organisms from animals to humans as a major form of threat to human health. Based on the aforementioned reports and in conformity with past trends of pandemic diseases and monitoring, it can be inferred that an effective approach to mitigate pandemics is the development of enhanced pathogen detection systems to prevent the spread of diseases irrespective of the source. Notably, two distinctive groups of pathogenic detection techniques have been reported in literature for clinical diagnosis; the conventional/traditional cell culture technique and the use of analytical devices (biosensors). The traditional methods chiefly rely on specific microbiological and biochemical detection via plated cultures. These methods are time consuming and labour intensive, although they are inexpensive and can yield both qualitative and quantitative results (Lee *et al.*, 2008; Velusamy *et al.*, 2010). Molecular

detection methods in the form of biomarking and biosensing are receiving attention as viable replacements to traditional cell plating methods. These methods can be engineered to have optimal performance variables such as high sensitivity, reliability, rapidity, specificity and simplicity in use (Fukushima *et al.*, 2007; Gracias and McKillip, 2004; Leonard *et al.*, 2003; Velusamy *et al.*, 2010). Despite the introduction of molecular biosensing technologies for high sensitivity detection and monitoring of pathogenic species, there still exists major drawbacks that hinder applications for routine mass monitoring, screening and evaluation exercises.

Laboratories and clinical centres have adopted molecular detection mechanisms based on polymerase chain reaction (PCR) to amplify specific nucleic acid sequences of pathogens for identification. Commercially available rapid identification tests use PCR followed by reverse hybridization to distinguish between pathogenic species. The technique is faster and more specific than cell colonies identification but the cost of these kits for routine use and mass evaluation is prohibitive, and pathogen identification can take a couple of days (Green *et al.*, 2009; Nahid *et al.*, 2014). Immunological detection using antibodies is also a commonly used advanced technique successfully employed for the detection of pathogens. This mechanism has been boosted by the development of hybridoma and antibody display technologies (Leonard *et al.*, 2003). Whilst PCR-based techniques offer a higher specificity, immunological detection is faster. Nonetheless both techniques do not offer rapid real-time detection essential for mass evaluation exercises, and as such these technologies are unfit for applications requiring almost instantaneous results (Leonard *et al.*, 2003).

Bioaffinity sensing formats can be used for direct detection of pathogens without the need for prior biochemical treatment of the sample. It requires the design of molecular probes with high specificity towards target species, and presently, antibodies are the most established probes for bioaffinity detection. However, the significant effort required to produce highly specific antibodies towards a single target is a major challenge towards the use of antibody probes (Velusamy *et al.*, 2010), and this has triggered the need for alternative biomolecular probes. Systematic evolution of ligands by exponential enrichment (SELEX), an iterative selection and amplification mechanism, can be used to

generate nucleic acid aptamers which are short single-stranded RNA or DNA sequences that can selectively bind to specific biomolecular or cellular targets, and have a wide range of biomedical applications. Molecular binding occurs via interaction between the target and the 3-D loop structure of the aptamer (Guo *et al.*, 2008). Aptamers can be engineered to demonstrate desirable biosensing characteristics such as high specificity, rapid detection, high sensitivity, easy to read, non-reactive, and high performance under physiological conditions with an infinite spectrum of potential targets (Radom *et al.*, 2013; Santosh and Yadava, 2014). Aptamers can retain their binding characteristics after immobilisation and can be tagged with different functionalities. Cell-based SELEX, targeting whole living cells, can be used to generate specific aptamers with high affinity towards membrane receptors or surface proteins, serving as the optimal molecular probe to accurately detect and characterise the pathogen at the molecular level (Guo *et al.*, 2008). Thus, this article discusses specific applications of aptameric sensing for pathogen detection as an effective approach to mitigating pandemics.

2.0 GLOBAL PANDEMIC OUTBREAK AND MONITORING

Pandemic diseases are characterised by specific epidemiological features such as wide geographic extension, swift disease movement, high attack rates and explosiveness, minimal population immunity, novelty, contagiousness and severity (Morens *et al.*, 2009). WHO aims at predicting future disease outbreaks before it reaches pandemic levels. This will enable rapid deployment of resources to control the spread of the disease. Table I presents a list of past, present and potential pandemic diseases. As a result of epidemiological studies and vaccination, some past pandemic diseases, such as plague, small pox and typhus, have been mitigated or eradicated. However, pandemic diseases such as HIV/AIDS and Cholera are still trending on especially in developing countries. Presently, the Department of Pandemic and Epidemic Diseases of the World Health Organisation has identified 16 diseases as potential pandemic causing diseases of the 21st century. These are Avian influenza, Cholera, Plague, Emerging diseases (such as nodding disease), Leptospirosis, Nipah virus infection, Viral hepatitis (A, B, C, E), Influenza (seasonal and pandemic), Viral haemorrhagic fevers (such as

Ebola, Marburg, Lassa, and Crimean-Congo haemorrhagic fever), Tularaemia, Rift Valley fever, SARS and coronavirus infections, Meningitis, yellow fever and Hendra virus infection (www.who.int/csr/disease/en/).

3.0 PATHOGEN DETECTION FOR PANDEMIC MONITORING

There exists a wide range of known and unknown pathogenic environments by which access to a healthy human host emanates on contact. These include water, food, agricultural, clinical samples, domestic and wild animals, and these materials are essential in identification and monitoring of any pandemic or potentially pandemic diseases. The most common mode of transmission during pandemic outbreaks is through contact with body fluids/sites of suspected infected humans and aerosols. The detection and identification of emerging or re-emerging pandemic pathogens is crucial according to WHO, hence rapid detection and identification of such pathogens will enhance the monitoring of pandemic cases since they are inevitable (Morens *et al.*, 2009). Successful detection of first cases will help in the identification of the source and mode of transmission.

The traditional cell culture and colony counting method is regarded as the standard method for pathogen detection and identification (Leland and Ginocchio, 2007; Wark *et al.*, 2010). It is performed *in vitro* in a controlled and defined environment through the isolation of cells from the tissues of animals or plants, involving a series of fastidious culturing and sub-culturing of microorganisms and biochemical recognition. The standard protocol involves a 16mm x 25mm plastic or glass round-bottom screw-cap tube for multiple detections (Leland and Ginocchio, 2007), though the results can be very slow. Shell vial and microwell plate, on the other hand, are comparatively faster (Leland and Ginocchio, 2007). Wolun-Cholewa *et al.* (2013) reported on the development of a novel 3-D cell culture support from Polyaniline nanostructured 3D grids. Halldorsson *et al.* (2015) also discussed the pros and cons of novel microfluidic devices intended to improve the cell culture technique. A detailed comparison between various developments in the cell culture technique is presented in Table II. With the aforementioned limitations of cell culturing, most clinical laboratories

are investing in molecular techniques as the standard methods for pathogen detection for rapid diagnosis and prognosis of patients (Hodinka and Kaiser, 2013).

3.1 Molecular Techniques for Detection and Identification

The development of molecular techniques are intended to overcome issues relating to the complicated growth profile of some pathogenic cells such as mycobacterium tuberculosis; the need to detect pathogenic microorganisms with low numbers; and lastly the need to detect emerging and re-emerging highly infectious micro-organisms (Fawley and Wilcox, 2005). Various kinds of molecular diagnostic techniques are in existence. However, the most predominant molecular technique in clinical diagnosis for pandemic diseases is the PCR technique. In general, the principle behind molecular technique is reliant on the detection, identification, characterization and manipulation of specific sequences of nucleotides or unique surface proteins of the pathogen (Wark *et al.*, 2010). The selection of an appropriate molecular technique is dependent on the following factors: nature of the sample, cost, simplicity in use and the ease of data interpretation (Monis and Giglio, 2006).

DNA based molecular techniques rely on either the sensing capacity of non-amplified DNA probes or through nucleic acid amplification. DNA probes, usually used in diagnostic kits, are designed to be complementary to specific nucleic acid sequences of the pathogen under stringent conditions (Riahi *et al.*, 2011; Wu *et al.*, 2013). To detect the hybridised nucleic acid probe, reporter molecules can be labelled on the probe to exhibit detectable and measurable features. The reporter molecules may be enzymatic, chemiluminescent, antibodies or fluorescent dyes (Wu *et al.*, 2013). Some established molecular techniques involving the application of non-amplified DNA probes for clinical diagnosis are shown in Figure I.

Nucleic acid amplification techniques (NAAT) are used for *in vitro* amplification of nucleic acids. Various NAAT exist for enhanced detection of pathogens based on their genome. PCR technique remains the most commonly used NAAT in research and clinical diagnosis of infectious and contagious diseases (She and Marlowe, 2013). PCR technique allows for the detection, identification and production of large amounts of specific pathogenic DNAs out of a large array with much rapidity

and sensitivity (Garibyan and Avashia, 2013; Loeffelholz and Deng, 2013). The entire *in vitro* cyclic process can be partitioned into three steps involving: (i) denaturing of the pathogenic DNA (ii) annealing of the separated DNA strands of the pathogen with the primers at a specified lowered temperature, and (iii) elongation of the primers through the addition of nucleotides to the developing DNA strand at specified elevated temperatures (Garibyan and Avashia, 2013; Loeffelholz and Deng, 2013). Various forms of PCR techniques are in existence for the detection of pathogens. These include conventional PCR, allele-specific PCR, hot-start PCR, touchdown PCR, degenerate PCR, multiplex PCR, nested and heminested PCR, reverse transcription-PCR, quantitative PCR and real-time PCR (Loeffelholz and Deng, 2013).

Herein, features of the various PCR techniques are briefly highlighted. Allele-specific PCR is used to detect and identify closely related species of bacteria. Hot-start PCR is used to increase the yield of target by reducing the activities of polymerase during the reaction set-up. Degenerate PCR enhances the detection of divergent sequences through the use of degenerate primers. Touchdown PCR enhances the reduction in the detection of non-specified products. Nested and heminested PCR improves sensitivity and specificity though they are associated with high cost and long duration. Multiplex PCR are useful for multiple detections of target DNA sequences. Reverse transcriptase-PCR, a qualitative technique based on the establishment of complementary DNA transcripts of targets from their RNAs, is mainly suitable for the diagnosis and prognosis of infectious diseases such as tuberculosis, HIV-1, and Avian influenza. Real-time PCR yields simultaneous periodic amplifications. Quantitative PCR is ideal for the quantification of nucleic acid targets (Loeffelholz and Deng, 2013).

Major milestones have been achieved in the development and application of PCR techniques for the detection of infectious diseases. Huq *et al.* (2012) reported the importance of sample purification in the detection of *Vibrio Cholera* species although this prolonged the assay time. Sample purification was essential for the elimination of organic matters capable of inhibiting amplification (Huq *et al.*, 2012). Ntema *et al.* (2010) also reported that pre-enrichment of samples before multiplex PCR (m-

PCR) improved the sensitivity: 4–10 cfu/100mL compared to 40-100cfu/100mL for non-enriched sample of *vibrios*. Optimal process variables for the application of PCR techniques have been developed to detect the pandemic strain *Vibrio parahaemolyticus* (Myers *et al.*, 2003). For viral pathogens, a pan haemagglutinin (PanHA) RT-PCR technique has been developed to identify variant influenza sub-types on site using five derived degenerated primers (Gall *et al.*, 2008). Also, an m-RT-PCR has been developed using a dual priming oligonucleotide system to detect H1N1, H1N2 and H3N2 subtypes at a low concentration of 1 $TCID_{50}$/ml per subtype (Lee *et al.*, 2008).

The merits of using PCR techniques include rapidity, high sensitivity, several multiple replication of trace DNA for qualitative and quantitative analysis (Garibyan and Avashia, 2013; Postollec *et al.*, 2011), quantification of detected DNA with qPCR (Garibyan and Avashia, 2013), capacity to detect viable but non-culturable pathogens (Postollec *et al.*, 2011), provision of information on microbial activity especially when qPCR is used in conjunction with reverse transcription (Postollec *et al.*, 2011), and less labour intensive compared to cell culture techniques. However, a number of challenges are faced in the application of PCR technique and this comprises of the potential for the inclusion of false results from trace contaminations due to high sensitivity of the technique (Garibyan and Avashia, 2013; Wark *et al.*, 2010), and the lack of capacity for applications relating to unknown pathogenic species since the technique relies on known data to develop primers (Garibyan and Avashia, 2013). This continues to remain a difficulty for the application of this technique to emerging and novel infectious pathogens. Also, primers used in PCR can anneal to themselves or to unwanted DNAs resulting in a false result (Garibyan and Avashia, 2013). During DNA sequencing, incorrect nucleotides can be included by the enzyme at low rates (Garibyan and Avashia, 2013). PCR is a costly and complex process thus requires the presence of trained personnel (Velusamy *et al.*, 2010).

3.2 Bioaffinity Techniques

Bioaffinity techniques that can be deployed in the mitigation and monitoring of pandemic diseases are reliant on the use of biomarkers. Biomarkers can be engineered to detect variant pathogens on a

molecular level, eliminating the high risk of trial and errors during diagnosis (Soontornworajit and Wang, 2011). The capacity for real-time biosensing using bioaffinity techniques is essential for pandemic monitoring.

Antibodies are predominantly used as probes for this technique in clinical diagnosis. A conventional method of employing antibodies is through the use of enzyme linked immunosorbent assays (ELISA). ELISA involves a series of steps reliant on antibodies-antigens affinity to detect the presence and quantity of microbial pathogens in a sample (Lazcka *et al.*, 2007). The antigens in this case are the target pathogens of interest, often immobilised in a 96 well plate. However, such techniques do not produce the much needed real-time results. On the other hand, immunochromatography assays which employ antibodies can be used for rapid detection of specific antigens, and this technique has been applied in detecting pandemic strains such as influenza virus (Mitamura *et al.*, 2013; Sakurai *et al.*, 2014), mycobacterium tuberculosis (Marzouk *et al.*, 2011; Park *et al.*, 2009) and HIV (Sacks *et al.*, 2012). A fast real-time result, within 15 minutes, has been achieved with immunochromatography. Despite successes in using immunochromatography for rapid clinical diagnosis, reports from medical experts during and after the 2009 pandemic H1N1 showed low sensitivities within a wide range of 10% to 70% (Ginocchio, 2011). This has retarded interest in the use of this technique. However, improvements in sensitivity by 10 fold have been reported by Sakurai *et al.* (2014) through the labelling of antibodies with coloured NanoAct beads for diagnosis and typing of influenza, and this can be adapted and applied to other diseases with pandemic strains for rapid detection (Sakurai *et al.*, 2014).

Owing to the inherent limitations of antibodies, setbacks in the area of sensitivity and specificity cannot be eliminated totally. Immunoassays are still faced with a number of challenges, albeit an improvement over the conventional and molecular techniques (Van Dorst *et al.*, 2010). The immobilisation of antibodies in a stable, high density and reproducible manner with no loss in bioactivity is very challenging. The significant effort required to produce highly specific antibodies towards a single target is another hindrance towards the use of antibody probes (Velusamy *et al.*,

2010). There are also ethical issues with the usage of animal parts for inexpensive production of polyclonal antibodies. It is impossible to use antibodies as the affinity element to detect cells or molecules that are immunogenic or toxic. Polyclonal antibodies are non-specific in their binding to specified targets, a challenge which is partially resolved through the use of hybridoma technology, though an expensive technique (Van Dorst *et al.*, 2010). Antibody elements cannot be used in austere environmental conditions, and are subject to contamination (Van Dorst *et al.*, 2010). These and many other challenges have led to the development of aptamer based affinity sensors, some of which have been successfully applied in several areas relating to biomedical research and medical applications (Radom *et al.*, 2013; Santosh and Yadava, 2014).

4.0 Aptamers as bioprobes

Aptamers are generated *in vitro* by means of a robust screening technique termed as SELEX from a large pool of synthesised oligonucleotide sequences (Tuerk *et al.*,1990). The library sequences are within the range of 10^{13}-10^{15}(de-los-Santos-Álvarez *et al.*, 2008; Radom *et al.*, 2013; Santosh and Yadava, 2014). The screening technique is made up of four systematic steps: variation, selection, partition and amplification. The process is repeated sequentially until the oligonucleotide sequence with the lowest affinity constant, K_d, binding to the target is selected from the library of oligonucleotides (Tuerk *et al.*,1990). Extensive reviews covering advances and modifications of SELEX have been reported previously (Aquino-Jarquin and Toscano-Garibay, 2011; McKeague and Derosa, 2012; Stoltenburg *et al.*, 2007). Table III compares the characteristics of aptamer recognition elements as biosensing probes with PCR molecular technologies to draw out their similarities and comparative advantages.

4.1 Aptamer immobilisation for rapid biosensing

Immobilisation of aptamers on solid supports is achieved through the chemical modification of its 3' or 5' end to incorporate functional groups that can enhance bonding to the support (de-los-Santos-

Álvarez *et al.*, 2008; Toh *et al.*, 2015). The chemistries involved in the immobilisation of aptamers on solid supports are reliant on the principles of covalent bonding, adsorption, affinity reactions and chemisorption (Bănică, 2012). This supports the development of label free biosensors, thus eliminating any interruptions from conjugated markers (de-los-Santos-Álvarez *et al.*, 2008; Xu *et al.*, 2005). Through immobilisation, aptamers are endowed with the ability for bio-detection as well as bioscreening of biomolecular and cellular targets. By virtue of their small size (in the range of 3000-20000 Da) as compared to antibodies, aptamer immobilisation on polymeric supports leads to a reduction in steric hindrance, large surface area coverage, and a high dense surface immobilisation for improved throughputs (Deng *et al.*, 2001). Immobilised aptamers can be denatured and regenerated continuously, and undergo repeated cycles of denaturing and renaturing (Luzi *et al.*, 2003; Van Dorst *et al.*, 2010).

Report by de-los-Santos-Álvarez *et al.* (2008) showed that the established affinity between aptamers and their targets can alter during the immobilisation process. This challenge is, however, suppressed through the use of spacers between the aptamer and the surface binding moiety (de-los-Santos-Álvarez *et al.*, 2008). The advantages of spacers are to help in reducing steric hindrance, maintain the shape of the aptamer, increase aptamer-target contact from the surface and minimise non-specific adsorption to the surface of the support (Balamurugan *et al.*, 2008; Zheng *et al.*, 2011). Figure II shows a spacer-arm linkage between an aptameric ligand and a support system. Examples of spacers include: polyethylene glycol (PEG), hexa-ethyloxy-glycol, oligonucleotides, alkyl chains, and mercaptoundecanoic acid (Chou *et al.*, 2004; Waybrant *et al.*, 2014; Zheng *et al.*, 2011).

Surfaces for aptamer immobilisation can be either 2-dimensional or 3-dimensional. Some examples of 2-dimensional surfaces include glass slide, poly(methyl methacrylate) substrates and silicon chips, whereas 3-dimensional surfaces include magnetic micro-spheres, agarose micro-spheres, silica micro-spheres, monoliths and polystyrene micro-spheres. An example of an immobilised aptamer on a 2–dimensional surface in a microarray format for pathogen detection is shown in Figure III below.

3-dimensional surfaces are more beneficial than 2-dimensional surfaces in terms of enhancing the performance of the aptamer after immobilisation. For an equivalent area for both supports, the surface area-to-volume ratio is higher for the latter. Owing to this, a conducive environment for aptamer-target interaction is provided as they are able to move more freely with their 3-D loop structures and conformations in the presence of the target (Sinitsyna *et al.*, 2012). Table IV compares 2-dimensional and 3-dimensional surfaces for aptamer immobilisation and biosensing.

Aptameric sensing using magnetic, agarose and silica micro-spheres have notably been used for screening targets such as cocaine and food related toxic compounds with high specificity and sensitivity (Chapuis-Hugon *et al.*, 2011; Madru *et al.*, 2011; Madru *et al.*, 2009; Wu *et al.*, 2011). Despite the achievement of this great milestone, major drawbacks of this configuration include the slow diffusive mass transfer of samples, the small size of the inter-particle space, and the possible existence of void fractions. An effective approach could rely on the use of continuous phase macroporous polymers, such as monoliths, for high throughput, specific, sensitive and rapid screening of pandemic pathogenic (viruses and bacteria) species (Danquah and Forde, 2008; Jungbauer and Hahn, 2008; Podgornik and Krajnc, 2012; Podgornik *et al.*, 2013).

Justifications to the use of macroporous polymers include: (i) enhanced mode of transfer by convective mass transport; (ii) good chemical and mechanical stabilities, pores interconnectivities with reduced pressure drop; (iii) ease of pore size control to handle different size of pathogens without clogging, retention of immobilised ligand density for continual usage; (iv) and the possible miniaturisation to portable sizes to enable the development of rapid diagnostic kits (Chan *et al.*, 2014; Danquah *et al.*, 2008; Podgornik *et al.*, 2013; Roberts *et al.*, 2009; Svec, 2010). It has recently been reported that monolithic adsorbents have a fast analysis time and can offer high throughput isolation and analysis of large protein molecules, DNA, cells, viruses and virus-alike particles in a chromatographic context. (Podgornik and Krajnc, 2012; Podgornik *et al.*, 2013). The sample-containing target is introduced into the monolithic aptasensor by means of a syringe for disk adsorbents or by high pressure application for column adsorbents. The high affinity between the

aptamer-functionalised adsorbent and the target causes specific binding which can be exploited for analytical purposes pathogenic identification, titration, screening via elution using UV, pH or conductivity analyses to obtain real time results on a chromatograph. The real-time results evaluate the difference in characteristics between the sample-containing target and the target-free sample exiting the column (Deng *et al.*, 2012). As the bond system between aptameric ligands and monolithic supports is largely covalent (Mallik and Hage, 2006), it enables the retention of aptamers to prevent ligand leaching during target elution with chaotropic reagents (Jungbauer and Hahn, 2008). This robustness bodes well for prolonged reusability and affordability for extended usage. A schematic of the standard procedure for high throughput operation using monolithic aptasensors is shown in in Figure IV. In brief, monolithic systems are gradually gaining grounds as appropriate supports for an all embedded realistic aptamer-biosensors for high throughput and instantaneous results in different configurations based on the above-mentioned features (Brothier and Pichon, 2014; Deng *et al.*, 2012; Han *et al.*, 2012; Zhao *et al.*, 2008). A summary of specific milestones covered for the immobilisation of aptamers on monoliths for bioseparation and purification applications is also presented in Table V.

4.2 Mass Screening with Immobilised Aptamer Sensors

The essence of biomedical screening during pandemics is to detect the presence of pathogens or associated biotoxins in people during or before symptomatic stages. It also helps in identifying and isolating both human and non-human hosts of the pathogen. Probes used for this purpose should be highly sensitive to detect low concentration levels of the pathogen in order to break the chain of transmission. Unfortunately, most rapid diagnostic kits are incapable of detecting the presence of target pathogens in asymptomatic individuals harbouring low pathogen levels. For instance, during the 2009 pandemic H1N1, infrared scanners were used to distinguish between symptomatic and asymptomatic individuals (Sakaguchi *et al.*, 2012). However, there were instances where the status of asymptomatic patients could not be confirmed after coming into close proximity with infected patients. Also, the deployment of rapid diagnostic kits with a sensitivity of 53.5% frequently yielded

false results especially in the early days of sample collection from individuals (Sakaguchi *et al.*, 2012).

Aptamers with their unique three-dimensional structures, alongside being short and single stranded, enable them to bind against an infinite pool of biological targets with much rapidity, sensitivity and specificity, differentiating between different types of pandemic strains. Owing to the variation in size of targets and number of binding sites, generally two modes of aptamer-target configuration exist. These are the single-site binding and dual-site binding (sandwich assay). The latter is displayed by targets small in size and the former by targets large in size (Song *et al.*, 2008). Label free signals from aptamer-target interactions can be captured through optical, mass sensitive and electrochemical means (Song *et al.*, 2008). Sensors developed based on optical signals are noted for their selectivity and sensitivity (Arora *et al.*, 2011; Lazcka *et al.*, 2007; Velusamy *et al.*, 2010). Examples of measured optical signals include but not limited to reflection, fibre optics, Raman, refraction, dispersion, fluorescence, infrared, chemiluminescence, and phosphorescence (Velusamy *et al.*, 2010). However, their associated cost and complexity are prohibitive (Lazcka *et al.*, 2007). Electrochemical-based aptasensors, like other sensors, can be categorised into amperometric, impedimetric, conductometric and potentiometric. They are an easy to use format of sensors and economical, but with poor selectivity and sensitivity for pathogen detection (Lazcka *et al.*, 2007). Hence, undesirable for rapid detection of pathogens. Aptasensors developed based on the differential change in mass are sensitive, specific, less complex, economical and can be made suitable for pathogenic detection. The differential mass change that occurs when the aptamer binds to the target is detected by means of piezoelectricity generated from an increase in oscillation on the surface of a crystal such as quartz (Velusamy *et al.*, 2010). For labelled aptasensors, the choice of labels is critical to avoid interference with the binding mechanism of the aptamer towards its target (de-los-Santos-Álvarez *et al.*, 2008).

Tertiary structures of aptamers formed after binding to targets include a hair pin, G-quartet, stem-bulge, combination, pseudoknot and T-junction structures (de-los-Santos-Álvarez *et al.*, 2008). Aptameric binding is characterised by the three dimensional structure of the aptamer and the sequence

(Van Dorst *et al.*, 2010). Unlike antibodies, aptamers can be synthesised *in vitro* towards specific targets with an avoidance of batch to batch variations, chemically modified to enhance stability, resistant to a wide variety of buffer changes, and can easily distinguish between enantiomers (Van Dorst *et al.*, 2010). The benefits of aptamers have heightened research interests in its applications in diagnostic devices (de-los-Santos-Álvarez *et al.*, 2008). Aptameric sensing can detect pathogens even in their latent state, and samples can be tested without pre-treatment as compared to molecular techniques and immunoassays (Lim *et al.*, 2005).

4.3 An outlook on aptameric sensing for pathogen detection

The ease by which aptamers bond to their targets gives room for their incorporation in rapid diagnostic kits (Minunni *et al.*, 2004). The unique binding features of aptamers imply that they can be used in lieu of immunochromatography assay, ELISA and, immunobead assay for rapid detection and screening (Minunni *et al.*, 2004; Toh *et al.*, 2015). The application of aptamers as point of care test kits with a reported time of below 15 minutes has been successfully developed for influenza A targets (Kiilerich-Pedersen *et al.*, 2013). The system works on the principle of variations in impendence resulting from the binding between the target and the immobilised aptamer. The technique is also highly specific, stable and with detection limit below clinical requirements (Kiilerich-Pedersen *et al.*, 2013).

Several other studies have been done and milestones covered in regards to developing aptamer sensors for virulent diseases. Rotherham *et al.* (2012) reported on the use of DNA aptamers for the detection of CFP-10.ESAT-6 heterodimer typical with *Mycobacterium tuberculosis*. Sensitivity and specificity levels of 100% and 68.75% were attained using Youden's index, whereas 35% and 95% were obtained using a rule-in cut-point method, respectively (Rotherham *et al.*, 2012). Minunni *et al.* (2004) reported on the feasibility of developing aptasensors in comparisons to immunobased sensors to probe HIV-1 TAT protein. Parameters such as specificity, reproducibility and reusability were studied by functionalising aptamers on a gold electrode of piezoelectric quartz-crystals. Notably, a

vast difference was observed when a non-treated aptamer was compared to a thermally treated aptamer prior to immobilisation and interaction with the analyte, HIV-1 TAT protein, yielding an average frequency shift from 19± 2Hz to 63±10Hz and a reproducibility, in terms of coefficient of variation, from 10% to 16% (Minunni *et al.*, 2004). Ruslinda *et al.* (2013) demonstrated the potential and reusability of a RNA aptamer immobilised on a diamond field effect transistor for the detection of real HIV-1 Tat protein samples at concentrations as low as 1nM. A streptavidin DNA aptamer has also been studied for the detection of interferon-gamma at an optimised detection limit of 33pM (Chang *et al.*, 2012). A bi-functional protein binding aptamer-DNA functionalised on a quantum dot has been described by means of Fluorescent Polarisation (FP) for the detection of H1N1 influenza A sequences (Zhang *et al.*, 2013). The detection limit for the reported aptamer recognition technique was 3.45 nM with a specificity value of about 48FP/mP (Zhang *et al.*, 2013). Wang *et al.* (2015b) reported on the development of a DNA aptamer-based bifunctional bio-nanogate and was validated with an H5N1 avian influenza virus as the target in a sample consisting of H1N1, H2N2, H4N8 and H7N2 as non-targets. The reported detection limit within an hour for the label free bio-nano gate aptasensors was 2^{-9} HAU without any matrix effect. In addition, a linear predictor equation was developed for viral targets (titre range of 2^{-10} and 2^{-2} HAU) with high selectivity for the cognate target, H5N1(Wang *et al.*, 2015b).

4.4 Current setbacks in deploying aptamer technologies

In spite of the superior qualities, aptamers possess as bioaffinity ligands, only little achievements have been realised in real-life applications. General insights into the challenges affecting the use of aptamers for the development of rapid biosensors are discussed in this section. One of such setbacks arises during the SELEX process for the generation of aptamers from oligonucleotides. In brevity, the setbacks in the SELEX process include the formation of sequence overlaps; the selection of nucleic acid strands with the appropriate orientation after the cyclic amplification step; and the possible development of concatamers (Radom et al., 2013). Nevertheless, these challenges can be

resolved by means of pre-and/or post-SELEX modification processes (McKeague and Derosa, 2012; Radom *et al.*, 2013).

According to Baird (2010), although significant body of knowledge has been generated from academic and research point of view, less is known of aptamers in the mainstream industry by practitioners. Current research work is mostly focused on generating aptamers and understanding the fundamental binding theories of aptameric binding with limited emphasis on the development of aptameric biosensors for real-life application. Furthermore, there is the expected resistance to change from the tried and tested antibody assays to aptameric assays despite the reported drawback of the later. A PubMed search of the term "aptamer assay" in 2010 revealed 939 articles by Baird (2010) but has increased exponentially to over 2600 as at present. Yet the situation has not drastically changed for real-life application in detecting pathogens. Consequently, there is no real-life application of aptameric technologies for any of the recent severe epidemics and pandemics over the past decade despite the general increase in aptamer research. Research covering aptamer application has largely been geared towards thrombin assays, possibly due to the ease of generation and availability of the thrombin aptamer. This has been tagged as the "thrombin problem" by researchers (Baird, 2010).

There is currently no approved standard for the application of aptamers for virulent and pandemic strains of pathogens, and this is a major hindrance to the development and routinized application of aptameric technologies for pathogen detection. However, Pegaptanib aptamer, though not related to pathogenic species, has received FDA approval, with a host of other aptamers under clinical trials for specific applications (Anthony *et al.*, 2010; Santosh and Yadava, 2014).

Wang *et al.* (2015a) highlighted that most aptasensors are faced with the challenge of reusability for more than 15 times stemming from improper immobilisation and regeneration protocols which leads to skewed orientation and loss of binding sites**.** Nevertheless, recently developed aptamer-based evanescent wave all-fiber biosensors on 3-D magnetic beads have been demonstrated to encompass

the propensity of being reused for more than 300 times whiles maintaining acceptable sensitivities (Wang *et al.*, 2015a).

4.5 Economic Viability of Aptamer Technologies

With conventional pathogen detection assays such as cell culture and molecular detection challenged with contamination, cost, delayed and false results, the use of aptameric sensing has become promising considering the technological and socio-economic benefits. The *in vitro* generation and specificity of aptamers alongside the ability to detect latent pandemic pathogens and immunogenic targets enable sustained use of aptamers for pathogenic detection. The use of polymerase chain reaction to produce aptamers with high reusability potential whiles maintaining their efficacy (Baird, 2010; Wang *et al.*, 2015a) indicates their suitability for rapid mitigation of pandemic pathogens at an affordable cost. The analysis and turn-around time involved with the deployment of aptamer technologies is significantly less compared to antibodies (Chen and Yang, 2015), and can be used without sample preparations and pre-culturing steps. It is expected that the global market value of aptamers may increase to about $2.1 billion dollars with an increase in the number of companies involved in the commerce by 2018 (http://www.marketsandmarkets.com/PressReleases/aptamers-technology.asp). This will potentially lead to a further drop in the cost of aptamer technologies as competition arises to make them more affordable.

5.0 Conclusion

Bearing the fact that pandemics and severe outbreaks are inevitable, with detrimental effects on every aspects of the society, there is an urgent need to invest into technologies that are effective and affordable.

Research advances in the application of aptamer technologies demonstrate that rapid detection of disease pathogens with much specificity, sensitivity, reproducible results and low detection limits can be achieved. Aptameric sensing can be used for pathogenic screening at entry and exit points of

geographical territories to curtail transmission between two locations. It can be applied not only for epidemic and pandemic diseases but also for endemic diseases.

A major drawback to the full scale application of aptamer technologies lies in the standardization and acceptance of aptameric sensing protocols as a more versatile, specific and efficient technology to replace and/or complement molecular techniques, immunoassays, and phenotypic methods of detecting pathogen in clinical samples. In addition, generation of effective chemistries on3-D supports in-lieu of the notable 2-D supports for aptamer immobilisation will further enhance target detection, reduce sample volume and cost, increase throughput, and promote the design of portable kits for domestic pathogen detection during pandemics. Extensive biophysical studies of the support-aptamer-target interactions are also required to achieve this endeavour.

6.0 Acknowledgment

The authors wish to thank the Ministry of Higher Education (Malaysia) and Curtin Sarawak Research Institute for providing the financial support for this research through the Fundamental Research Grant Scheme (FRGS) and Curtin Flagship schemes respectively. Authors wish to declare that there is no conflict of interest or commercial with respect to the publication.

7.0 REFERENCES

1. Anthony DK, Supriya P, Andrew E. Aptamers as therapeutics. Nature Reviews Drug Discovery. 2010;9:660.
2. Aquino-Jarquin G, Toscano-Garibay JD. RNA aptamer evolution: Two decades of selection. International journal of molecular sciences. 2011;12:9155-9171.
3. Arora P, Sindhu A, Dilbaghi N, Chaudhury A. Biosensors as innovative tools for the detection of food borne pathogens. Biosensors & bioelectronics. 2011;28:1-12.
4. Baird GS. Where are all the aptamers? American journal of clinical pathology. 2010;134:529-531.
5. Balamurugan S, Obubuafo A, McCarley RL, Soper SA, Spivak DA. Effect of linker structure on surface density of aptamer monolayers and their corresponding protein binding efficiency. Analytical chemistry. 2008;80:9630-9634.
6. Bănică FG. Nucleic Acids in Chemical Sensors. Chemical Sensors and Biosensors: Fundamentals and Applications. West Sussex, U.K: John Wiley & Sons Inc; 2012. p. 118-134.

7. Brothier F, Pichon V. Miniaturized DNA aptamer-based monolithic sorbent for selective extraction of a target analyte coupled on-line to nanoLC. Analytical and bioanalytical chemistry. 2014;406:7875-7886.

8. Chan AS, Hartley PG, Zhu Y, Danquah MK, Agyei D. A Parametric Study of a Monolithic Microfluidic System for On-Chip Biomolecular Separation. Separation Science and Technology (Philadelphia). 2014;49:854-860.

9. Chang CC, Lin S, Lee CH, Chuang TL, Hsueh PR, Lai HC, *et al.* Amplified surface plasmon resonance immunosensor for interferon-gamma based on a streptavidin-incorporated aptamer. Biosensors & bioelectronics. 2012;37:68-74.

10. Chapuis-Hugon F, du Boisbaudry A, Madru B, Pichon V. New extraction sorbent based on aptamers for the determination of ochratoxin A in red wine. Analytical and bioanalytical chemistry. 2011;400:1199-1207.

11. Chen A, Yang S. Replacing antibodies with aptamers in lateral flow immunoassay. Biosensors and Bioelectronics. 2015;71:230-242.

12. Chou CC, Chen CH, Lee TT, Peck K. Optimization of probe length and the number of probes per gene for optimal microarray analysis of gene expression. Nucleic acids research. 2004;32:e99.

13. Danquah MK, Forde GM. Preparation of macroporous methacrylate monolithic material with convective flow properties for bioseparation: Investigating the kinetics of pore formation and hydrodynamic performance. Chemical Engineering Journal. 2008;140:593-599.

14. Danquah MK, Ho J, Forde GM. A thermal expulsion approach to homogeneous large-volume methacrylate monolith preparation; Enabling large-scale rapid purification of biomolecules. Journal of Applied Polymer Science. 2008;109:2426-2433.

15. de-los-Santos-Álvarez N, Lobo-Castañón MaJ, Miranda-Ordieres AJ, Tuñón-Blanco P. Aptamers as recognition elements for label-free analytical devices. TrAC Trends in Analytical Chemistry. 2008;27:437-446.

16. Deng N, Liang Z, Liang Y, Sui Z, Zhang L, Wu Q, *et al.* Aptamer modified organic-inorganic hybrid silica monolithic capillary columns for highly selective recognition of thrombin. Analytical chemistry. 2012;84:10186-10190.

17. Deng Q, German I, Buchanan D, Kennedy RT. Retention and separation of adenosine and analogues by affinity chromatography with an aptamer stationary phase. Analytical chemistry. 2001;73:5415-5421.

18. Fawley WN, Wilcox MH. Molecular diagnostic techniques. Medicine. 2005;33:26-32.

19. Fukushima H, Katsube K, Hata Y, Kishi R, Fujiwara S. Rapid separation and concentration of food-borne pathogens in food samples prior to quantification by viable-cell counting and real-time PCR. Applied and environmental microbiology. 2007;73:92-100.

20. Gall A, Hoffmann B, Harder T, Grund C, Beer M. Universal Primer Set for Amplification and Sequencing of HA_0 Cleavage Sites of All Influenza A Viruses. Universal Primer Set for Amplification and Sequencing of HA_0 Cleavage Sites of All Influenza A Viruses. 2008;46:2561-2567.

21. Gao C, Sun X, Woolley AT. Fluorescent measurement of affinity binding between thrombin and its aptamers using on-chip affinity monoliths. Journal of chromatography A. 2013;1291:92-96.

22. Garibyan L, Avashia N. Polymerase chain reaction. The Journal of investigative dermatology. 2013;133:e6.

23. Ginocchio CC. Strengths and weaknesses of FDA-approved/cleared diagnostic devices for the molecular detection of respiratory pathogens. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;52 Suppl 4:S312-325.

24. Gracias KS, McKillip JL. A review of conventional detection and enumeration methods for pathogenic bacteria in food. Canadian journal of microbiology. 2004;50:883-890.

25. Green C, Huggett JF, Talbot E, Mwaba P, Reither K, I. ZA. Rapid diagnosis of tuberculosis through the detection of mycobacterial DNA in urine by nucleic acid amplification methods. Lancet Infect Dis. 2009;9:505-511.

26. Guo K, Paul A, Schichor C, Ziemer G, Wendel HP. CELL-SELEX: Novel Perspectives of Aptamer-Based Therapeutics. . Int J Mol Sci 2008; 9:668-678.

27. Halldorsson S, Lucumi E, Gomez-Sjoberg R, Fleming RM. Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. Biosensors & bioelectronics. 2015;63:218-231.

28. Han B, Zhao C, Yin J, Wang H. High performance aptamer affinity chromatography for single-step selective extraction and screening of basic protein lysozyme. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2012;903:112-117.

29. Hodinka R, Kaiser L. Is the Era of Viral Culture Over in the Clinical Microbiology Laboratory? J Clin Microbiol. 2013;51:2-8.

30. Huq A, Haley BJ, Taviani E, Chen A, Hasan NA, Colwell RR. Detection, isolation, and identification of Vibrio cholerae from the environment. Current protocols in microbiology. 2012;Chapter 6:Unit6A 5.

31. Jungbauer A, Hahn R. Polymethacrylate monoliths for preparative and industrial separation of biomolecular assemblies. Journal of chromatography A. 2008;1184:62-79.

32. Kiilerich-Pedersen K, Dapra J, Cherre S, Rozlosnik N. High sensitivity point-of-care device for direct virus diagnostics. Biosensors & bioelectronics. 2013;49:374-379.

33. Lazcka O, Del Campo FJ, Munoz FX. Pathogen detection: a perspective of traditional methods and biosensors. Biosensors & bioelectronics. 2007;22:1205-1217.

34. Lee CS, Kang BK, Lee DH, Lyou SH, Park BK, Ann SK, *et al.* One-step multiplex RT-PCR for detection and subtyping of swine influenza H1, H3, N1, N2 viruses in clinical samples using a dual priming oligonucleotide (DPO) system. Journal of virological methods. 2008;151:30-34.

35. Leland DS, Ginocchio CC. Role of cell culture for virus detection in the age of technology. Clinical microbiology reviews. 2007;20:49-78.

36. Leonard P, Hearty S, Brennan J, Dunne L, Quinn J, Chakraborty T, *et al.* Advances in biosensors for detection of pathogens in food and water. Enzyme and Microbial Technology. 2003;32:3-13.

37. Lim DV, Simpson JM, Kearns EA, Kramer MF. Current and developing technologies for monitoring agents of bioterrorism and biowarfare. Clinical microbiology reviews. 2005;18:583-607.

38. Loeffelholz M, Deng H. PCR and its variations. Advanced Techniques in Diagnostic Microbiology: Springer; 2013. p. 166-183.

39. Luzi E, Minunni M, Tombelli S, Mascini M. New trends in affinity sensing: aptamers for ligand binding. Trac-Trends Anal Chem. 2003;22:810-818.

40. Madru B, Chapuis-Hugon F, Pichon V. Novel extraction supports based on immobilised aptamers: evaluation for the selective extraction of cocaine. Talanta. 2011;85:616-624.

41. Madru B, Chapuis-Hugon F, Pichon V, Peyrin E. Determination of cocaine in human plasma by selective solid-phase extraction using an aptamer-based sorbent. Analytical chemistry. 2009;81:7081-7086.

42. Mallik R, Hage DS. Affinity monolith chromatography. Journal of separation science. 2006;29:1686-1704.

43. Marzouk M, Kahla IB, Hannachi N, Ferjeni A, Salma WB, Ghezal S, *et al.* Evaluation of an immunochromatographic assay for rapid identification of Mycobacterium tuberculosis complex in clinical isolates. Diagnostic microbiology and infectious disease. 2011;69:396-399.

44. McKeague M, Derosa MC. Challenges and opportunities for small molecule aptamer development. Journal of Nucleic Acids. 2012;2012.

45. Minunni M, Tombelli S, Gullotto A, Luzi E, Mascini M. Development of biosensors with aptamers as bio-recognition element: the case of HIV-1 Tat protein. Biosensors & bioelectronics. 2004;20:1149-1156.

46. Mitamura K, Kawakami C, Shimizu H, Abe T, Konomi Y, Yasumi Y, *et al.* Evaluation of a new immunochromatographic assay for rapid identification of influenza A, B, and A(H1N1)2009 viruses. Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy. 2013;19:633-638.

47. Monis PT, Giglio S. Nucleic acid amplification-based techniques for pathogen detection and identification. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2006;6:2-12.

48. Morens DM, Folkers GK, Fauci AS. What is a pandemic? The Journal of infectious diseases. 2009;200:1018-1021.

49. Myers ML, Panicker G, Bej AK. PCR Detection of a Newly Emerged Pandemic Vibrio parahaemolyticus O3:K6 Pathogen in Pure Cultures and Seeded Waters from the Gulf of Mexico. Applied and environmental microbiology. 2003;69:2194-2200.

50. Nahid P, Bliven-Sizemore E, Jarlsberg LG, De Groote MA, Johnson JL, Muzanyi G, *et al.* Aptamer-based proteomic signature of intensive phase treatment response in pulmonary tuberculosis. Tuberculosis. 2014;94:187-196.

51. Ntema VM, Potgieter N, Barnard TG. Detection of Vibrio cholerae and Vibrio parahaemolyticus by molecular and culture based methods from source water to household container-stored water at the point-of-use in South African rural communities. Water science and technology : a journal of the International Association on Water Pollution Research. 2010;61:3091-3101.

52. Park MY, Kim YJ, Hwang SH, Kim HH, Lee EY, Jeong SH, *et al.* Evaluation of an immunochromatographic assay kit for rapid identification of Mycobacterium tuberculosis complex in clinical isolates. Journal of clinical microbiology. 2009;47:481-484.

53. Peterson L, Hamilton JD, Baron E, Tompkins L, Miller J, Wilfert CM, *et al.* Role of clinical microbiology laboratories in the management and control of infectious diseases and the delivery of health care. Clin Infect Dis. 2001;32:605-610.

54. Pike B, Saylors K, Fair J, Lebreton M, Tamoufe U, Djoko C, *et al.* The Origin and Prevention of Pandemics. Clin Infect Dis. 2010;50:1636-1640.

55. Podgornik A, Krajnc NL. Application of monoliths for bioparticle isolation. Journal of separation science. 2012;35:3059-3072.

56. Podgornik A, Yamamoto S, Peterka M, Krajnc NL. Fast separation of large biomolecules using short monolithic columns. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2013;927:80-89.

57. Postollec F, Falentin H, Pavan S, Combrisson J, Sohier D. Recent advances in quantitative PCR (qPCR) applications in food microbiology. Food microbiology. 2011;28:848-861.

58. Radom F, Jurek PM, Mazurek MP, Otlewski J, Jelen F. Aptamers: molecules of great potential. Biotechnology advances. 2013;31:1260-1274.

59. Riahi R, Mach KE, Mohan R, Liao JC, Wong PK. Molecular detection of bacterial pathogens using microparticle enhanced double-stranded DNA probes. Analytical chemistry. 2011;83:6349-6354.

60. Roberts MW, Ongkudon CM, Forde GM, Danquah MK. Versatility of polymethacrylate monoliths for chromatographic purification of biomolecules. Journal of separation science. 2009;32:2485-2494.

61. Rotherham LS, Maserumule C, Dheda K, Theron J, Khati M. Selection and application of ssDNA aptamers to detect active TB from sputum samples. PloS one. 2012;7:e46862.

62. Ruslinda RA, Tanabe K, Ibori S, Wang X, Kawarada H. Effects of diamond-FET-based RNA aptamer sensing for detection of real sample of HIV-1 Tat protein. Biosensors & bioelectronics. 2013;40:277-282.

63. Ryan JR, Glarum JF. Pandemic Influenza Emergency Planning and Community Preparedness. Boca Raton, U.S.A.: Taylor and Francis Group; 2008.

64. Sacks R, Omodele-Lucien A, Whitbread N, Muir D, Smith A. Rapid HIV testing using Determine HIV 1/2 antibody tests: is there a difference between the visual appearance of true- and false-positive tests? International journal of STD & AIDS. 2012;23:644-646.

65. Sakaguchi H, Kawashima M, Yoshino Y, Tsunoda M, Aizawa Y, Wada K, *et al.* Assessment of border control measures and community containment measures used in japan during the early stages of Pandemic (H1N1) 2009. PloS one. 2012;7.

66. Sakurai A, Takayama K, Nomura N, Yamamoto N, Sakoda Y, Kobayashi Y, *et al.* Multi-colored immunochromatography using nanobeads for rapid and sensitive typing of seasonal influenza viruses. Journal of virological methods. 2014;209:62-68.

67. Santosh B, Yadava PK. Nucleic acid aptamers: research tools in disease diagnostics and therapeutics. BioMed research international. 2014;2014:540451.

68. She RC, Marlowe EM. Non-PCR Target Amplification Techniques. Advanced Techniques in Diagnostic Microbiology: Springer; 2013. p. 293-306.

69. Sinitsyna ES, Walter JG, Vlakh EG, Stahl F, Kasper C, Tennikova TB. Macroporous methacrylate-based monoliths as platforms for DNA microarrays. Talanta. 2012;93:139-146.

70. Song S, Wang L, Li J, Fan C, Zhao J. Aptamer-based biosensors. TrAC Trends in Analytical Chemistry. 2008;27:108-117.

71. Soontornworajit B, Wang Y. Nucleic acid aptamers for clinical diagnosis: cell detection and molecular imaging. Analytical and bioanalytical chemistry. 2011;399:1591-1599.

72. Stoltenburg R, Reinemann C, Strehlitz B. SELEX—A (r)evolutionary method to generate high-affinity nucleic acid ligands. Biomolecular Engineering. 2007;24:381-403.

73. Svec F. Porous polymer monoliths: Amazingly wide variety of techniques enabling their preparation. Journal of Chromatography A. 2010;1217:902-924.

74. Thomas JC, Lavender MG. Pandemic Influenza Emergency Planning and Community Preparedness. Boca Raton, U.S.A.: Taylor and Francis Group; 2008.

75. Toh SY, Citartan M, Gopinath SCB, Tang T-H. Aptamers as a replacement for antibodies in enzyme-linked immunosorbent assay. Biosensors and Bioelectronics. 2015;64:392-403.

76. Van Dorst B, Mehta J, Bekaert K, Rouah-Martin E, De Coen W, Dubruel P, *et al.* Recent advances in recognition elements of food and environmental biosensors: a review. Biosensors & bioelectronics. 2010;26:1178-1194.

77. Velusamy V, Arshak K, Korostynska O, Oliwa K, Adley C. An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnology advances. 2010;28:232-254.

78. Wang R, Xiang Y, Zhou X, Liu L-H, Shi H. A reusable aptamer-based evanescent wave all-fiber biosensor for highly sensitive detection of Ochratoxin A. Biosensors and Bioelectronics. 2015a;66:11-18.

79. Wang R, Xu L, Li Y. Bio-nanogate controlled enzymatic reaction for virus sensing. Biosensors and Bioelectronics. 2015b;67:400-407.

80. Wark AW, Lee J, Kim S, Faisal SN, Lee HJ. Bioaffinity detection of pathogens on surfaces. Journal of Industrial and Engineering Chemistry. 2010;16:169-177.

81. Waybrant B, Pearce T, Kokkoli E. Effect of Polyethylene Glycol, Alkyl, and Oligonucleotide Spacers on the Binding, Secondary Structure, and Self-Assembly of Fractalkine Binding FKN-S2 Aptamer-Amphiphiles. Langmuir : the ACS journal of surfaces and colloids. 2014;30:7465-7474.

82. Wolun-Cholewa M, Langer K, Szymanowski K, Glodek A, Jankowska A, Warchol W, *et al.* An efficient 3D cell culture method on biomimetic nanostructured grids. PloS one. 2013;8:e72936.

83. Wu F, Hong T, Della-Latta P. Nonamplified Probe-Based Microbial Detection and Identification. Advanced Techniques in Diagnostic Microbiology: Springer; 2013. p. 227-237.

84. Wu X, Hu J, Zhu B, Lu L, Huang X, Pang D. Aptamer-targeted magnetic nanospheres as a solid-phase extraction sorbent for determination of ochratoxin A in food samples. Journal of chromatography A. 2011;1218:7341-7346.

85. Xu D, Xu D, Yu X, Liu Z, He W, Ma Z. Label-free electrochemical detection for aptamer-based array electrodes. Anal Chem. 2005;77:5107-5113.

86. Zhang J, Tian J, He Y, Chen S, Jiang Y, Zhao Y, *et al.* Protein-binding aptamer assisted signal amplification for the detection of influenza A (H1N1) DNA sequences based on quantum dot fluorescence polarization analysis. The Analyst. 2013;138:4722-4727.

87. Zhao Q, Li X-F, Le XC. Aptamer-modified monolithic capillary chromatography for protein separation and detection. Analytical chemistry. 2008;80:3915-3920.

88. Zheng R, Cameron BD, Park B-W, Kim D-S. Development of a highly specific amine-terminated aptamer functionalized surface plasmon resonance biosensor for blood protein detection. Biomedical optics express. 2011;2:2731-2740.

Figure captions

Fig 1 This figure shows the general trend in developing rapid techniques for pathogen detection from cell cultures to aptameric recognition

Fig. 2 Illustration of spacer applications between modified-aptamer and the moiety of the adsorbent matrix to offer a better aptamer positioning for effective target interactions

Fig 3 Illustration of a microarray system using a 2-D coated substrate with immobilised aptamers

Fig 4 A continuous stationary phase with immobilised aptamers for rapid sensing and convective adsorption of target molecules

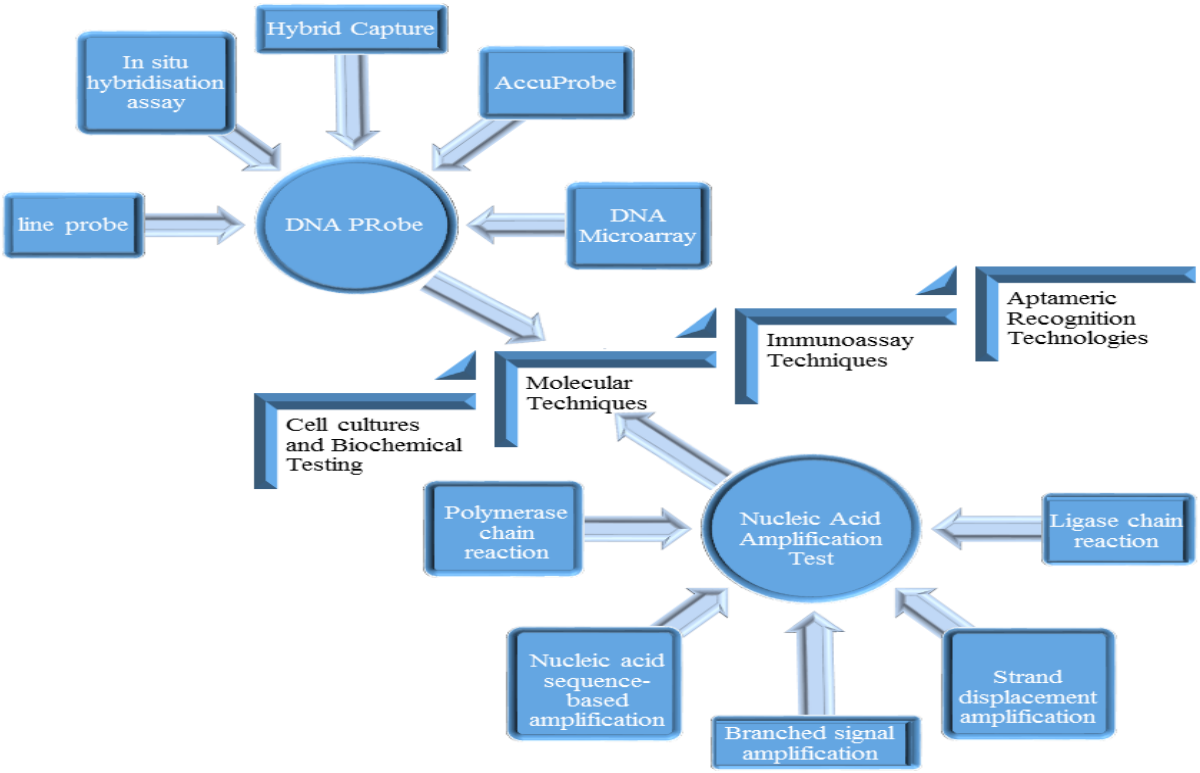

Fig 1 This figure shows the general trend in developing rapid techniques for pathogen detection from cell cultures to aptameric recognition

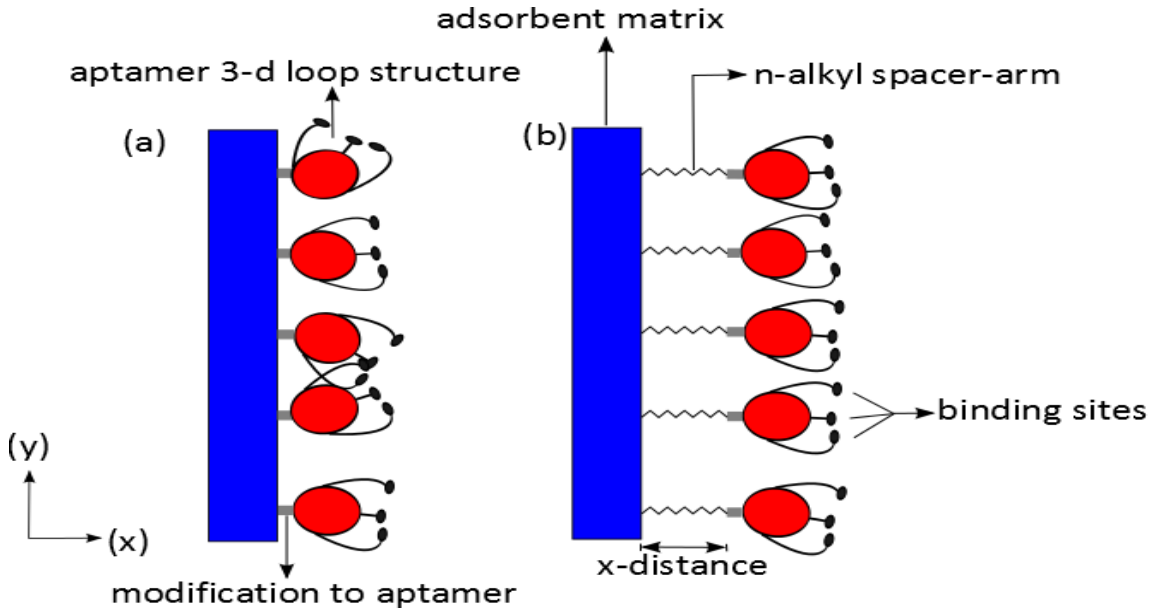

Fig. 2 Illustration of spacer applications between modified-aptamer and the moiety of the adsorbent matrix to offer a better aptamer positioning for effective target interactions

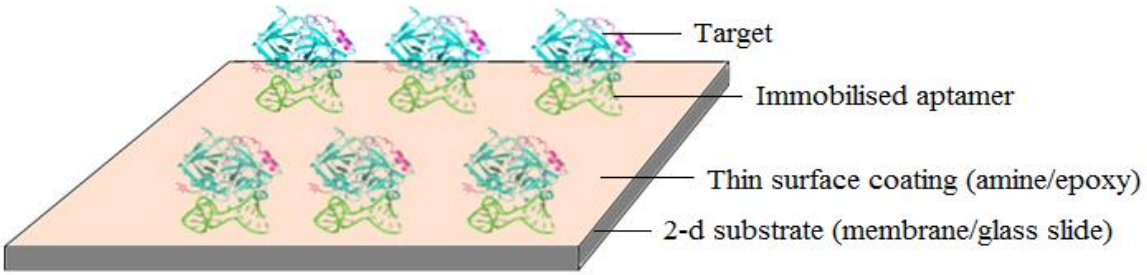

Fig 3 Illustration of a microarray system using a 2-D coated substrate with immobilised aptamers

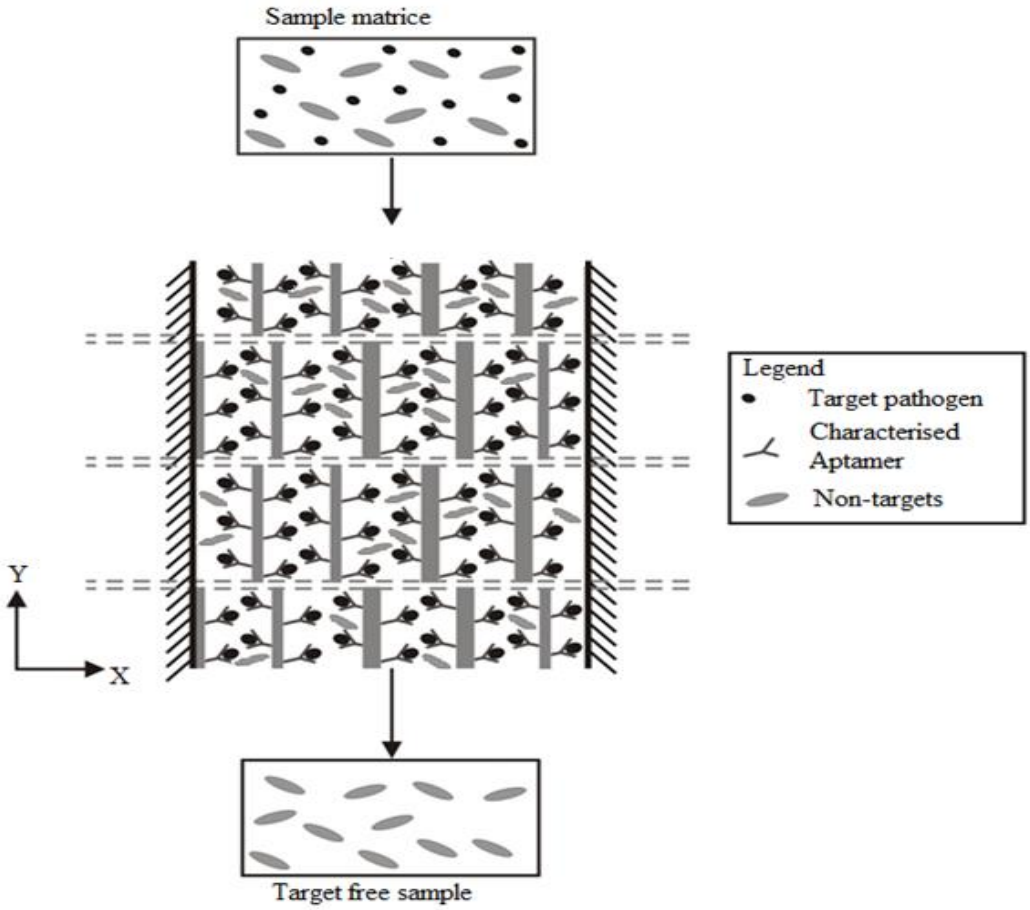

Fig 4 A continuous stationary phase with immobilised aptamers for rapid sensing and convective adsorption of target molecules

Tables

Table I. Past, present and potential pandemic diseases

Classification	Pathogens with known pandemic strains	Number of known recorded Pandemics	Remarks
Past Pandemics	*Variola major* and *Variola minor*	1	Named pathogen associated with Small Pox. Totally Eradicated in 1979.
	Rickettsia bacteria	1	Causative agent for Typhus. It is believed to have been eradicated but there are reported cases currently with different emerging strains of the bacteria (Edouard et al., 2014)
	H3N2	1	Eradicated with no recorded outbreak presently
	H1N1	3	The virus continues to emerge from zoonotic sources
	H2N2	1	Eradicated with no recorded outbreak presently
	M. Tuberculosis	1	No longer a pandemic disease but possesses a high infection and mortality rate.
	Plague bacillus (*Yersinia pestis*)	3	The plague disease has been mitigated. However, there arethousands of reported cases globally. Mostly

			dominant in Africa with potential higher number of infections (Stenseth et al., 2008)
Current Pandemics	HIV-1	1	The pandemic strain HIV-1 is still in transmission without cure.
	Vibrio Cholera	7	The seventh pandemic strain is still in transmission with reported epidemic outbreaks in some developing countries.
	Ebola Viral		According to the WHO, the worst epidemic form of EBOLA is still trending on in West Africa with a few scattered cases in other continents, and fears of it mutating if active measures are not put into effect. Current average case fatality ratio is about 50%.
Future Possible Pandemics	H5N1		Resulting from possible viral mutation and the current resistance to antibiotics by some pathogenic species.
	Viral Haemorrhagic fevers such as Ebola		
	SARS-Coronavirus		
	M. Tuberculosis		

Table II. Characteristics of various cell culture technologies to enhance pathogen detection.

Cell culture supports	Advantages	Disadvantages	Reference(s)
Traditional ware	Can be used for multiple detections, proliferations are essential in diagnostic decisions, possess excellent sensitivity.	Requires technical expertise; culturing of pathogens is time and labour intensive; viable but non-culturable cells, such as for vibrio cholera species, will yield false results; costly; requires huge infrastructure set up during pandemics.	(Leland and Ginocchio, 2007, Hodinka and Kaiser, 2013, Velusamy et al., 2010)
Shell vial	Faster detection than traditional ware; viral proliferations are used in taking diagnostic decisions; excellent sensitivity; and less susceptible to contamination	Requires technical expertise; culturing of pathogens is time and labour intensive; viable but non-culturable cells, such as for vibrio cholera species, will yield false results; costly; requires huge infrastructure set up during pandemics.	(Leland and Ginocchio, 2007, Hodinka and Kaiser, 2013, Velusamy et al., 2010)
Microtiter plate	A more rapid detection than traditional ware; viral proliferations are utilised in diagnostic decisions; and excellent sensitivity.	Susceptible to cross-contamination; requires technical expertise; culturing of pathogens is time and labour intensive; viable but non-culturable cells will yield false results; costly; require huge infrastructure set up during pandemics; and lacks real time output.	(Leland and Ginocchio, 2007, Velusamy et al., 2010)
Microfluidic cell culture	Real time cell division imaging with high resolution; automatic plating of cells culturing, harvesting and	No standard protocol exists yet; requires technical expertise; long duration to obtain confirmed results due to	(Halldorsson et al., 2015)

	replenishing processes; capable of mimicking the natural environment of cells; temperature stable micro-environment; high throughput; does not require highly expensive incubators; and less intake of reagents.	sample preparation and incubation.	
Electrospun nanostructured fibers	Relatively high surface to volume ratio; can withstand overcrowding growth load of culture; transparent scaffolds for real time observation; standard fluorescence or colorimetric methods can be employed for evaluation of cells; and the support can be embedded in *in situ* conditions for ultra-structural studies.	Requires technical expertise; long duration to get confirmed results due to sample preparation, slow growth rate of some organisms and incubation; and cannot be used to study viable but non-culturable cells.	(Wolun-Cholewa et al., 2013)

Table III. Juxtaposition of PCR and aptamer recognition technologies.

Parameter	Molecular Diagnostic Technology (PCR)	Aptameric Recognition Technology	Reference(s)
Recognition element	Mostly natural nucleic acids	Synthetic nucleic acids	
Target Space	Has a limited number of targets (Unable to detect protein toxins and non-nucleic acid targets)	Can be generated for an almost unlimited number of targets	(Lim et al., 2005, Radom et al., 2013)
Nucleic acid characterisation	Highly susceptible to contamination	Less susceptible	(Garibyan and Avashia, 2013, Santosh and Yadava, 2014)
Sample preparation	Compulsory	Not essential	(Wark et al., 2010, Lim et al., 2005)
Rapidity	Fast but not comparable to aptamers	Rapid and real time	(Leonard et al., 2003, Garibyan and Avashia, 2013)
Economics	Costly	Less costly	(Velusamy et al., 2010, Santosh and Yadava, 2014)
Specificity	High	High	(Wark et al., 2010)
Sensitivity	High	High	(Garibyan and Avashia, 2013, Yang and Rothman, 2004)
Multiplexing	Applicable	Applicable	(Loeffelholz and Deng, 2013, de-los-Santos-Álvarez et al., 2008)
Assay	Label dependent	Optional depending on the format	(Wark et al., 2010, de-los-Santos-Álvarez et al., 2008)
Complexity	Requires trained technicians	Not required depending on the format	(Velusamy et al., 2010, Lazcka et al., 2007)

Point-of-care	Applicable	Applicable	(Baron and Campbell, 2013, Cass and Zhang, 2011)
Food and Drugs Authority protocols	Standard protocols set in place for numerous targets, especially infectious pathogens	No standard protocols have been set in place yet except for the macugen aptamer	(Yang and Rothman, 2004, Van Dorst et al., 2010)

Table IV. Comparison between 3-dimensional and 2-dimensional surfaces for immobilised aptameric biosensing.

Parameter	2-dimensional	3-dimensional
Loading capacity	Has a low loading capacity	Has a high loading capacity
Steric Hindrance	Highly susceptible to steric hindrance from adjacent aptamers due to low loading capacity	Less susceptible to steric hindrance due to high loading capacity
Assay	Comparably slow assay development	Quick assay development, a criterion of importance for pandemic application
Multiplexing	Can be used for multiplex assay	Highly efficient for multiplexing
Economics	Less economical	Highly economical
Sensing ability	Can be used only for detection	Can be used for both detection and adsorption of pathogens into the support matrix
Throughput	Has a low throughput	Has a high throughput

Table V. Milestones covered for the application of aptamer-immobilised monoliths for bio-separation and purification

Type of monolith	Characterised Aptamer	Description of monolith	Experimental function	Coverage density of immobilised aptamer (pmol/μL)	Selective retention time of target (min)	Reference
GMA-co-TRIM	61-mer DNA aptamer	Polymeric rod-like monoliths	For the separation of cytochrome c and thrombin from a sample of mixed biomolecules	164	2.4	(Zhao et al., 2008)
Organic−inorganic hybrid silica	29-mer DNA aptamer	Monolithic capillary columns	Selective enrichment of thrombin in complex biological fluids	568	*~(12-14)	(Deng et al., 2012)
poly(GMA-co-EDMA)	anti-Lys DNA aptamers	Polymeric rod-like monoliths	Selective screening of lysozyme (lys) from chicken egg white	290	13.43	(Han et al., 2012)
GMA-co-PEGDA	15-mer thrombin-binding aptamer	On-chip monolithic columns with PEG incorporated	Real-time florescent detection and separation of thrombin binding to an immobilised characterised aptamer in a complex mixture of biomolecules	Not specified	~3	(Wolun-Cholewa et al., 2013)
Hybrid organic-inorganic monolith	DNA aptamer	Monolithic capillary column	Selective extraction of Ochratoxin A from beer samples mixed with a binding buffer	6.27 nmol/μL	~9.91	(Brothier and Pichon, 2014)

**Glycidyl methacrylate (GMA); trimethylolpropanetrimethacrylate (TRIM); Poly Ethylene Glycol Dimethacrylate (PEGDA)