Advance detection technologies for select biothreat agents

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Introduction

Biological warfare agents are a unique class of weapons that pose dangers to all biodiversity; the future threat is directly linked to the technological advancement in modern biotechnology (Prockop, 2006; Atlas, 2002). The armed forces and the civilians are under constant threat from a variety of microorganisms that can be used as weapons of mass destruction (Christophe et al., 1997). Advanced biological warfare agents will pose the greatest challenge to the development of appropriate medical countermeasures. Meeting this challenge will require an effective biodefense strategy in terms of a robust biodefense program that delivers the diagnostic technologies, medicines, and vaccines needed to counter the range of advanced bioweapons of the 21st century.

Biological weapons are characterized by low visibility, high potency, substantial accessibility, and relatively easy delivery (Klietmann and Ruoff, 2001). Bioterrorism is the intentional use of microorganisms—bacteria, viruses, fungi, and toxins—to produce disease and death in humans, livestock, and/or crops (Eneh, 2012). The bio agents could be carried by winds, insects, or birds, none of which respect national borders. It thus becomes very difficult to detect the biological agent or to determine whether the victim has been deliberately infected (Noah et al., 2002; Riedel, 2004). The potential spectrum of bioterrorism ranges from hoaxes and use of non-mass casualty agents by individuals or small groups to state-sponsored terrorism that employs classic biological warfare (BW) agents and can produce large-scale outbreaks and mass casualties (Centers for Disease Control and Prevention, 1999). Such scenarios would present serious challenges for patient treatment and for prophylaxis of exposed persons. Further, environmental contamination could pose continuing threats. Harmfulness or hazardous (threat) of biological agents like Nipah, Ebola, Anthrax, Plague, etc., has increased significantly with the involvement of non-state actors who would exploit not only for mass casualties, but also for a variety of other purposes from the strategic to the tactical, leading to huge economic loss and general social disruption (Szinicz, 2005; Eitzen, 2001). Public awareness of the growing

threat of bioterrorism is gaining momentum all over the world. Thus there is an increased demand for overall preparedness to address the challenges pertaining to the diagnosis, treatment, and prophylaxis of new and re-emerging maliciously incited infectious diseases (Snowden, 2008; Grundmann, 2014). Anti-bioterrorism measures depend on the rapid biomonitoring of the situation as a part of pre-warning for immediate implementation of proper control measures (Parnell et al., 2010).

Bio-detection technologies

The threat of attack on military and civilian targets employing chemical and biological weapons is a growing national concern. Technologies for detection of these materials in the natural environment are being developed worldwide. While several technologies show great promise as broadband detectors, there is no silver bullet that detects all biological agents at the requisite levels of sensitivity and specificity.

Capability for detecting and identifying multiple biological warfare agents quickly and accurately is required to protect both troops on the battlefields and civilians confronted with terrorist attacks. The main focus of biodefense is therefore to develop fast, sensitive, automatic technologies for the detection and identification of biological warfare agents with a high degree of selectivity, sensitivity, and specificity (Lim et al., 2005). The detection technologies therefore focus on a variety of technologies including surface properties, genomic signatures, proteomics, etc. Ideally, a platform should be portable, easy to use, and capable of detecting multiple agents simultaneously. Platforms that integrate sample processing will have the benefit of reduced complexity for the operator. The sample processing method should be applicable for all sample types and all target analytes. In addition, instrumental techniques are widely used to detect toxins that are not amenable for DNA-based assays. The systems currently available for sensing biological analytes rely primarily on two technologies: reporter molecules that attach to antibodies and give off fluorescent signals, and the molecular PCR technology that amplifies suspect DNA. Because two steps are required to identify biological weapons, the procedure is both labor- and time-intensive (Huang et al., 2012; Martin et al., 2003).

Rapid, early, and accurate detection is the cornerstone in preventing loss of life and further spread of a disease leading to an epidemic in a biothreat scenario. From a preparedness perspective, early detection and response are crucial to minimize the potential consequences (Bravata et al., 2004). Systems to detect bioterrorism agents in clinical and environmental samples and to diagnose bioterrorism-related illnesses are essential components of responses to both hoaxes and actual bioterrorism events. The ability to identify rapidly the introduction of a bioterrorist agent into the civilian population will require highly sensitive, specific, inexpensive, high-throughput, and easy-to-use diagnostic tools (Peruski and Peruski, 2003; Rotz and Hughes, 2004). Ideally, these tests could also evaluate the possible spectrum of antimicrobial resistance and be connected to a central database. Centralized confirmatory testing also should be expanded to include routine evaluations of positive samples for genetic

profiling, and bioengineered properties. The theoretical ability to design and develop such assays exist, e.g., microchip-based platforms, which could contain thousands of microbial signature profiles that are either nucleic acid or protein based, providing standards for validation and comparison of potential products (Lillehoj et al., 2010; Ewalt et al., 2001). The scope of the chapter is to give an updated comprehensive review about technological developments happening in the field of biothreat agent detection.

Culture

The culture of a microbial agent has long been considered a gold standard of diagnosis. However, owing to several limitations including cost, time, expertise, and containment facility, alternate culture independent methods are now being explored. These methods offer better sensitivity and are capable of simultaneous detection of multiple agents, even novel pathogens. Further, the rapidity of the culture independent assay is critical for decision making, particularly in a biothreat scenario (Doggett et al., 2016; Hong et al., 2013).

Immunological assays

Enzyme linked immunosorbent assay (ELISA)-based systems are generally widely used for diagnosis of single microbial infection. Recent advancements using the Luminex xMAP technology offer multiplex capability (Reslova et al., 2017). The MagPix assay system based on ELISA principles is centered on paramagnetic microsphere technology, which can transition ELISAs to a more sensitive and consistent system with the added capability of multiplexing. It employs color-coded magnetic microspheres with antibodies covalently coupled to the beads as the solid support for an ELISA-like sandwich immunoassay. A charged coupled device (CCD) camera is used for detecting the fluorescence from each microsphere excited by light-emitting diodes and facilitates measuring the median fluorescence intensity (MFI) of each sample. The MFI is then used as the basis for sample analysis (Yan et al., 2017; Andreotti et al., 2003).

Immunochromatographic test (ICT)

The assay system is based on the lateral flow/flow-through principle, employing the colloidal gold as the indicator. The ICT system has revolutionized the field of immunodiagnosis by offering an easy-to-perform test at the patient's bedside, providing results in 5–10 min.

Lateral flow rapid strip test

Lateral flow tests are also called immunochromatographic tests (ICT). They have been a popular platform for rapid tests since their introduction in the late 1980s.

ICTs are used for the specific qualitative or semi-quantitative detection of many analytes including antigens, antibodies, and even the products of nucleic acid amplification tests. One or several analytes can be tested for simultaneously on the same strip. Urine, saliva, serum, plasma, or whole blood can be used as specimens. Extracts of patient exudates or fluids have also been used successfully. Lateral flow immunoassay (LFIA) are easy to perform and fast, but they are not too sensitive and give more false-positive results. However, they may be useful for rapid initial screening of samples for the presence of biological agents, although, as a matter of principle, any positive result must be confirmed by other tests, such as PCR. Lateral flow devices have been developed by many companies for large number of biological agents as *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, *Clostridium botulinum*, and several toxins, such as ricin and staphylococcal enterotoxin B (Cox et al., 2015; Li et al., 2015; Gessler et al., 2007).

Flow through spot test

The test principle involves a flow of fluid containing the analyte through a porous membrane and into an absorbent pad. A second layer, or sub-membrane, inhibits the immediate back-flow of fluids, which can obscure results. These tests can be used to detect both antibodies and antigens. To detect antibodies and antigens, the corresponding analyte is bound or immobilized as a dot or line on the membrane. This reagent "captures" the analyte as it flows through the membrane. To perform the test, a sample is applied to the membrane and allowed to wick through by capillary action. Thereafter, sequentially, there is a wash step, addition of the signal reagent, and a second wash to clear the membrane. Recent flow-through tests have successfully used colored latex particles or colloidal gold. This is a very rapid test procedure (3–5 min). However, these are not "walk-away" tests as the lateral-flow test is. Test sensitivity is good for serological assays, but for solid-phase tests, detection of antigens is often less sensitive than lateral-flow or enzyme immunoassays (EIA) methods (Fan, 1991).

In response to the 2001 anthrax cases, considerable interest was generated in the handheld antibody-based detection tests such as the Sensitive Membrane Antigen Rapid Test (SMART) and the Antibody-based Lateral Flow Economical Recognition Ticket (ALERT) (Bravata et al., 2004). Such systems use antibodies to recognize specific targets on the toxins, antigens, or cells of interest. Limitations of these tests include nonspecific binding of the antibodies, which may lead to false-positive results, and degradation of the antibodies over time, which may lead to false-negative results. Additionally, these tests are limited by the availability of antibodies.

Molecular assays

Molecular detection methods rely on the unique nucleic acid (DNA/RNA) signature of a biological agent. These methods also tend to be more sensitive than antibody-based detection methods, with real-time PCR assays being able to detect 10 or fewer

microorganisms (Drosten et al., 2002). The major limitation of PCR is the inability to distinguish live and dead agents and multiplexing which is limited to 4–6 targets at the current time for real-time PCR. Much higher levels of multiplexing are possible with endpoint PCR methods using the Luminex system, but sensitivity, quantitative dynamic range, and specificity are reduced. A number of assay formats based on isothermal and non-isothermal are available and currently widely used for the gene amplification.

Polymerase chain reaction (PCR)

PCR remains the most popular and widely used technology. The major advantage of the nucleic acid-based system is its specificity due to the uniqueness of genome in living systems. The careful designing of primer and probes enables specific detection of organisms. It also offers the highest sensitivity due to exponential amplification of the genomic signature. During PCR, a short piece of genome (DNA/RNA) of the bio-threat agent is amplified, resulting in millions of copies of DNA within a short time (Towner et al., 2004; Mourya et al., 2012). A number of commercial versions of PCR systems are now available which consist of a disposable assay cartridge containing consumable reagents, an instrument that integrates the thermal components required for gene amplification, and the optical components required to quantify the amplified products (generally tagged with a fluorescent dye). Further, positive and negative controls are included as part of the assay to rule out reaction failure and validate both the assay and instrument performance. Sample preparation remains one of the important critical areas for the realization of on-site nucleic acid amplification systems. The results of PCR vary widely depending on the presence of inhibitors in the sample and are more crucial from complex environmental matrices, which require sample processing. Different modifications to the conventional PCR have been made that enable the simultaneous detection of multiple threat agents. The multiplex PCR substantially reduces cost and time (Nazarenko et al., 2002). The general contamination of source DNA in toxin samples is now exploited to identify the toxins.

Real-time RT-PCR

Compared to conventional end-point PCR, real-time (RT) PCR is quantitative in nature. It can simultaneously detect and quantify the nucleic acid in any sample. RT-PCR measures changes in fluorescence intensity which is proportional to the increase of the amplicon. RT-PCR are primarily of two types: specific and non-specific. In non-specific RT-PCR, a universal DNA interacting dye like SYBR Green is used that emits fluorescence when bound to DNA. Further, a melting curve analysis is performed following amplification that provides specificity based on the length and composition of amplicon. This format is simpler to perform and inexpensive due to requirement of only primers. The specific detection relies on the use of unique target genome-specific fluorogenic labeled probes. The increase in fluorescence indicates the hybridization of probes to the target DNA, leading to physical separation of the

fluorophore from the quencher (Liu et al., 2012). In contrast to non-specific SYBR Green format, probe-based assays provide multiplex capability through use of different fluorescent dyes. TaqMan Probes have been successfully employed for detection of multiple biothreat agents including *Bacillus anthracis*, *Yersinia pestis*, *Coxiella burneti*, Cat A biothreat viral agents including smallpox, Ebola, and other hemorrhagic viruses (Buzard et al., 2012).

Isothermal gene amplification assays

In contrast to RT-PCR, isothermal assays provide the advantage of a fast turnaround time. Isothermal loop-amplification (LAMP) is a novel powerful gene amplification technique that is widely adapted to early detection and identification of numerous microbial agents including biothreat pathogens. The isothermal nature makes this assay simple and rapid, and the whole amplification process can be completed within 1h. It employs a set of six specially designed primers spanning eight distinct sequences of a target gene, making the assay extremely specific. The gene amplification products can be detected by agarose gel electrophoresis as well as by real-time monitoring in an inexpensive turbidimeter. This assay is amenable to field application, as the amplified product can be visualized by the naked eye either as turbidity or in the form of a color change when a fluorescent dsDNA intercalating dye like SYBR Green I (Parida et al., 2011; Kurosaki et al., 2007).

Helicase-dependent amplification (HDA) is an isothermal amplification technology that closely mimics the PCR. However, it works in isothermal conditions due to the use of helicase capable of unwinding a DNA duplex. Recently, a novel isothermal real-time detection method (HDA-TaqMan) that combines the advantages of both HDA and a TaqMan assay was reported for detection of biothreat organisms: *Vibrio cholerae* and *Bacillus anthracis*. In this technique, the reactions of DNA unwinding, primer annealing, polymerization, probe hybridization, and subsequent hydrolysis by the polymerase are coordinated and synchronized to perform at a single temperature (Barreda-García et al., 2016).

Nucleic acid sequence-based amplification (NASBA) is another popular isothermal gene amplification technique used to detect viable organisms using mRNA as a template in both clinical and environmental matrix. In this method, the primer binds to the RNA target sequence, and reverse transcriptase produces a technique, a primer binds to mRNA and cDNA strand is generated. The parental RNA is then degraded by RNase. A second primer binds to cDNA, which is reverse transcribed and a double-stranded cDNA is synthesized. Finally, T7 RNA polymerase enzyme is used to synthesize RNA transcripts during the amplification process. This method has been applied to detect a number of pathogens including viruses, bacteria, fungi, and protozoa (Khaled et al., 2005; Birgit et al., 2002).

Understanding the dynamics of emerging and reemerging infections is critical to efforts to reduce the morbidity and mortality of such infections, to establish policy related to preparedness for infectious threats and for decisions on where to deploy limited resources against infection. Therefore there is a need for the creation of a

genome data bank for selected viral agents and circulating organisms in the country causing natural epidemics for reference and mapping. This will help to distinguish natural epidemics versus intentional biological warfare (BW) attack as well as to pinpoint the source and origin of the organism. Furthermore, this will also help to update the primer data bank and strategies for vaccine designing.

Next-generation sequencing (NGS) technology

NGS technology has the potential to revolutionize the field of molecular detection technology. This technology enables the sequencing of complete genome content of a clinical or environmental sample, creating a metagenome. This metagenome is not only capable of identification of known biothreat agent, but also can identify a hitherto unknown agent in a very short duration of time. NGS fills a vital role in characterization of disease outbreaks by whole-genome sequencing of isolates and in the identification of infectious agents when other diagnostics fail due to involvement of rare and novel pathogens. With suitable sample extraction technology that is compatible with a molecular detection platform, NGS can play a very important role in the rapid detection of agents from complex environmental matrix. A trade-off between yield and purity in the sample processing step can lead to efficient use of NGS technology. This technology has led to identification of a large number of novel species of non-culturable microbial and viral agents. The major limitation of NGS includes the high cost of instruments and complex interpretation of data (Karlsson et al., 2013; Gilchrist et al., 2015).

The focus of the nucleic acid sequence technology is the development of a biochip that contains an array of engineered molecules that react with the genome of biological warfare agents. The biochip is embedded in a platform that is portable, automated, and allows for direct sampling of the environment. A biochip platform to identify the anthrax bacteria is in the testing stages, and additional biochips for identifying other harmful bacteria and viruses are in development.

Biomonitoring

The most important step of the biodefence strategies is the rapid detection and identification of the causative agents. Detection is the unspecific demonstration of increased concentration of microorganisms in a particular environment, whereas identification is the species determination of the detected microorganism. The attack with BW agents is difficult to detect owing to the inherent intrinsic properties of the organisms. In cases of suspected use of BW agents, rapid detection and identification of the infectious agent are critical for early implementation of specific countermeasures. Therefore, the detection systems for BW agents should have the properties of rapidity, reliability, sensitivity, and specificity so as to diagnose quickly the correct etiological agent from complex environmental samples before the spreading of illness on a large scale (Lim et al., 2005).

The test systems that are suitable for the detection and identification of the agent in the laboratory cannot be directly applied for BW agents considering the nature of the environmental samples (air, water, soil, and foodstuffs). The environmental samples have highly complex structures and are therefore difficult to analyze. In addition, the concentration of the organism may be very low compared to the sensitivity or detection limit of the existing test systems. Therefore, it is essential to concentrate the microorganisms from the environmental samples in order to achieve the detectable concentrations prior to analysis.

Aerosol detection technologies

Real-time detection and measurement of biological agents in the environment are daunting. A myriad of microorganisms are present in the environment and each organism has its own signature. Most detection schemes are specific for a particular biological agent. Detection technologies are categorized by their requirement to come in direct physical contact with the biological agent. Depending on the need, detection system architecture and sensors involved will be different. For an early warning of a biological event, a "stand-off" detection system may be sufficient. For early warning systems, the sensitivity of the detection system is not important. The presence of live biological agents needs to be determined.

Air sampling of microorganisms is governed by the same principles of collection as other particulates; however, the viability of the organisms complicates their collection. The main objective is to keep the viability of the organism in a viable state so that subsequent identification steps become easier. The project envisages development of simpler and appropriate system for air sampling, analysis, and detection of biological agents in the aerosol/environment (Mary et al., 2003).

Sensor technologies

DNA array-based sensors: The DNA array class of detectors relies on comparing the DNA taken from microorganisms in a sample with the known DNA of known biowarfare agents. Researchers have in recent years been sequencing the DNA of an array of potential agents, using common gene sequencing methods (ESpehar-Délèze et al., 2015).

Protein array-based sensors: Multiplex protein liquid arrays will also be developed to detect more than one agent simultaneously. The system uses a liquid suspension array of sets of beads, each internally dyed with different ratios of two spectrally distinct fluorophores to assign it a unique spectral address. Each set of beads can be conjugated with different capture molecule. The conjugated beads can then be mixed and incubated with sample in a micro plate well to react with specific analytes. Captured molecules can be enzyme substrates, DNA, antigens, or antibodies. The bead array system can be used to detect several agents simultaneously (Birgit and Ehricht, 2006).

Immunological sensors: Although antibody technology has progressed steadily over the last five years with antibody biosensors becoming significantly smaller, such technology is still not truly man-portable and available in our country. It is proposed to develop antibody-based biosensors for environmental aerosol monitoring. One of the most sensitive designs relies on antibodies adsorbed onto the surface of colloidal gold particles, which gives a visual indication of the result (Karlsson et al., 1991).

Tissue-based biosensors: A drawback with the DNA and antibody tests is that they require prior knowledge of the bioagents. Many toxins, for example, trigger reactions in living cells. These reactions can be measured and differentiated. The tissue-based biosensor will be constructed with immobilized live cells which are seeded into a cartridge, and after exposure the response is measured. This is a completely new area of work and attempts will be made to initiate research in this new area (Wijesuriya, 1993).

MIP-based sensors: Molecular imprinting is a technique for the fabrication of bio-mimetic polymeric recognition sites or plastic antibodies/receptors which is attracting rapidly increasing interest. By this technology, recognition matrices can be prepared which possess high substrate selectivity and specificity. In the development of this technology, several applications have been foreseen in which imprinted materials may be exchanged for natural recognition elements. Thus MIPs have been used as antibody/receptor binding mimics in immunoassay-type analyses, as enzyme mimics catalytic applications and as recognition matrices in biosensors. Sensitive detection systems will be developed using MIPs against selected toxins (Selvolini and Giovanna, 2017).

Nanomaterials biosensors

Nanomaterials are efficient in addressing many of the limitations of existing sensors including speed, cost, mobility, and the stringent requirements of sample processing. Their small size and disposability make them excellent candidates for field-based sensors. As a substrate they provide high surface area on a platform that can be dispersed in an analytic sample and often provide feedback in less than a minute. Magnetic nanomaterials help in concentrating an analyte from a complex matrix and can also provide feedback even in opaque solutions. Quantum-confined semi-conducting nanomaterials possess photophysical properties that can be exploited for tagging analytes and participating in energy transfer, while their physical properties make them more durable than dyes and suited to a non-laboratory environment. The diversity of nanomaterials in conjugation with different assay formats is useful to create superior sensors (Clare et al., 2016).

Instrumental technologies
Mass spectrometry

The unique cellular fatty acid profile can be used effectively to identify the bio organism. Fatty acid analysis is more objective and less prone to human error.

Fatty acid analysis can identify to the strain level versus the species level for most DNA-based methods. It is proposed to develop initially GC-FID generated library comprising profiles of cellular fatty acids followed by generation of a library of fingerprint programs of available organisms. The data generated will be used to identify the potential toxins and bioagents in the environmental samples. The findings can be used as preliminary analytical data. The instrumental techniques to be used for generating fingerprints of toxins and bioagents include GC-MS, LC-MS, and MALDI-TOF (Boyer et al., 2015).

Mass spectrometry techniques have been widely reported following the development of soft ionization techniques (MALDI and ESI), and by the continuous development of MS technologies (high resolution, accurate mass HR/AM instruments, novel analyzers, hybrid configurations). Multiplexed toxin detection, discovery of new markers, and identification of untargeted novel molecular targets are successfully achieved. A proof of concept study has been reported for successful post-exposure recovery of biological agent in a simulated biothreat scenario using tandem mass spectrometry (Wang et al., 2014; Alam et al., 2012).

Toxins represent one of the most dreaded forms of bioterrorism agents and have been successfully employed for bioterrorism/biocrime events. Due to the proteinaceous nature of toxins, gene amplification assays are of limited utility. The detection of toxins relies mainly on immunological assays. However, proteomics approach based on MS/MS is sensitive, rapid, and allows absolute quantitation and multiplexing capabilities. Targeted LC-MS/MSA based assay was reported for specific detection and quantification of multiple toxins, namely ricin, *Clostridium perfringens* epsilon toxin (ETX), *Staphylococcus aureus* enterotoxins (SEA, SEB and SED), shigatoxins from Shigella dysenteriae, and entero-hemorragic *Escherichia coli* strains (STX1 and STX2) in complex food matrices. However, instrument cost and service contracts remain high, limiting developing countries use to large diagnostic laboratories.

PLEX-ID is a powerful technology that combines the power of both PCR and mass spectrometry (Murillo et al., 2013). PLEX-ID allows for rapid identification and genotyping of microorganisms including, bacteria, viruses, fungi, etc. Here initially either monoplex or multiplex PCR is carried out on the nucleic acid extracted from the sample. The amplicon is subjected to electrospray ionization and time-of-flight mass spectrometry that allows very accurate determination of molecular size and weight of both strands. Unique identification of the sample is possible through comparison with a reference database. There are a number of detection panels available now in this technology (respiratory virus, biothreat, broad bacteria, broad viruses, food-borne, multi-drug resistance, etc). The advantages of such technology include extremely high multiplexing capability (up to thousands of agents) and significant throughput. These features make PLEX-ID an excellent device in the case of analyzing samples of unknown origin.

Raman chemical imaging

Chemical imaging combines molecular spectroscopy and digital imaging, and has been demonstrated to be a powerful tool for the rapid molecular analysis of biological threat agents in complex matrices. Chemical imaging microscopy provides molecular composition and structural information, without the use of dyes or stains, at sub-micron spatial resolution, in a non-contact, non-invasive, reagent-less detection mode. Optical techniques for specimen interrogation include Raman scattering and fluorescence emission (Kathryn et al., 2007; Gregory et al., 2012).

Biodetectors

Biodetectors are analytical devices that combine the precision and selectivity of biological systems with the processing power of microelectronics. They act as powerful analytical tools in medicine, environmental diagnostics, and food industries, as well as forensic analysis and counterterrorism. Biodetectors usually consist of a biological recognition system, typically enzymes or binding proteins immobilized on a surface acting as a physicochemical transducer. One typical example of a biodetector is the immunosensor, which uses antibodies as the biorecognition system. In addition to enzymes and antibodies, the recognition systems can consist of nucleic acids, whole bacteria, and single-cell organisms, and even tissues of higher organisms. Specific interactions between the target molecule or analyte and the complementary biorecognition layer produce a detectable physico-chemical change, which can then be measured by the detector. The detection system can take many forms depending upon the parameters being measured. Electrochemical, optical, mass, or thermal changes are the most common parameters providing both qualitative or quantitative data. Electrochemical biosensors are promising platforms that could achieve rapid highly sensitive and selective on-site detection of such agents (Qian and Bau, 2004; Berchebru et al., 2014).

Commercially available biodetectors

A large number of commercial firms have developed a large number of detection systems for on-site detection of biothreat agents. These are based on either molecular or immunological detection platforms. These systems are highly suitable for handling by first responders. The immunological systems are based on immunochromatographic tests, which are rapid and result can be interpreted both on site as well as can be transmitted for offsite interpretation. The molecular detection platforms are based on both isothermal and real-time PCR. They provide high accuracy, sensitivity, and multiplexing capability to the detection platform. A list of advanced technologies based on molecular and immunological detection platform has been provided in Tables 1 and 2.

Table 1 List of PCR-based technologies for detection of biothreat agents

Sl. no.	Product name	Manufacturer	Principle	Run time	Sample preparation	Automatic result display	Freeze dried	No. of agents	
1	BioFire Defense, LLC: FilmArray	BioFire Diagnostics, Inc.; Biomerieux, France	Multiplex PCR	60 min	Minimal	Yes	Yes	16 agents (27 targets)	
2	Bio-Seeq PLUS	BioFire Diagnostics, Inc.; Biomerieux, France	LATE-PCR	60 min	Minimal	Yes	Yes	4 agents (5 targets)	
3	RAZOR EX	BioFire Defense, LLC, USA	RT-PCR	30 min	Minimal	Yes	Yes	10-agent assay	
4	one3	Biomeme, USA	PCR	60 min	Minimal	No	Yes	1-agent assay	
5	POCKIT	GeneReach Biotechnology Corporation, Taiwan	PCR	60 min	Moderate	Yes	Yes	1-agent assays	
6	POCKIT Micro Nucleic Acid Analyzer	GeneReach Biotechnology Corporation, Taiwan	Convection PCR	30 min	Moderate	Yes	Yes	1-agent assays	
7	T-COR 8	Tetracore, Inc. Rockville, USA	Multiplexed PCR	20–45 min	Minimal	Yes	Yes	2–3 agent assays	
8	FilmArray	BioFire, Salt Lake City, UT	Nested multiplex PCR	60 min	Minimal	Yes	Yes	16 biothreats	

LCCD, lowest concentration consistently detected; LATE-PCR, linear after the exponential polymerase chain reaction technology.

List of agents	Instrument cost	Assay cost	Detection limit	Wt	LCCD	Analyzing capacity	Shelf life of assay
1,2,3,4,5,6,7,8,9, 11,12,13,14,15	$39,500	$1110/6 pack	Different for different agents (in pdf)	20 lb	1000	Single sample for 27 targets	6 months
1,5,8,10,2,17, 3,18,9,16	$35,000	$46,199.18	100 organisms	6.6 lb	20,000	6 agents simultaneously	–
1,5,8,10,2, 17,3,18,9,16	$38,500	$768/64 reactions	100 cfu/mL	10 lb.	1000	single sample	6 months
1,9,19,16,20,3, 2,5,8,13,11,7, 21,6,22,12,23,24	$4950	$760 field kit	Different for different agents (in pdf)	1 lb.	100	Single sample	Five years
1,17,5 (5.1,5.2),9,25, 26,27	$8000	$380/48 reactions	Different for different agents (in pdf)	4.6 lb	2000	8 samples	24 months
1,17,5,5.1,5.2,9	$900	$380/48 reactions	Different for different agents (in pdf)	0.84 lb	10	8 samples	24 months
19,9,1,11, 16,6, 20,5,3,2	$28,500	$768/64 reactions	1–100 PFU per mL	10 lb.	2000	4 samples	12 months
28,29,30,31,17	$39,500	$3870	1.0E+04 CFU/mL	20 lbs	675	Single sample at a time	12 months

Table 2 List of immunoassays for detection of biothreat agents

S.no.	Product name	Manufacturer	Run time	Sample preparation	Automatic result display	No. of agents	
1	BADD	AdVnt Biotechnologies	~15 min	Minimal	Yes	6	
2	Pro Strips	AdVnt Biotechnologies, LLC	~15 min	Minimal	Yes	5	
3	RAID Multi-Test Strips	Alexeter Technologies, LLC	~15 min	Minimal	Yes	8	
4	NIDS assays and optical reader	ANP Technologies, Inc.	~15 min	Minimal	Yes	11	
5	IMASS assays	BBI Detection, LLC.	~15 min	No	Yes	7	
6	ENVI Assay System and optional reader	Environics, Inc.	~15 min	Minimal	Yes	4	
7	PR2 1800	Meso Scale Defense™	15–60 min	Moderate	Yes	16	
8	Smart II CANARY Zephyr	PathSensors, Inc.	~15 min	Minimal	Yes	6	
9	RAPTOR: Automated, Multianalyte Bioassay Detection System		~15 min	Minimal	Yes	10	
10	RAMP assays and optical reader	Response Biomedical Corp.	~20 min	Minimal	Yes	4	
11	BioThreat Alert assays and optical reader	Tetracore, Inc.	~15 min	Minimal	Yes	9	

Note: Bacillus anthracis-1, Francisella tularensis-2, Yersinia pestis-3, Clostridium botulinum-4, Brucella species-5 Brucella melitensis (5.1), Brucella abortus (5.2), Burkholderia mallei/pseudomallei-6, Ebola virus-7.1, MarBurg virus-7.2, Lassa virus-7.3, Coxiella burnetii-8, Ricin toxin-9, E. coli 157:H7-10, Variola virus-11, Rickettsia prowazekii-12, Venezuelan equine encephalitis virus-13, Eastern equine encephalitis virus-14, Western equine encephalitis virus-15, Plague-16, Salmonella-17, Small Pox-18, Abrin toxin (Abrus precatorius)-19, Staphylococcal Enterotoxin B (SEB)-20, Epsilon Toxin (Clostridium perfringens)-21, Chlamydophila psittaci-22, Vibrio cholerae-23, Cryptosporidium parvum-24, Dengue virus-25, Middle east respiratory syndrome coronavirus-26, Rift valley fever virus-27, Rotavirus-28, Campylobacter-29, Clostridium difficile-30, Norovirus-31, Shigatoxin-32, Viral Encephalitis-33, Glander-34, Malaria-35, Protozoan infection-36, Q-fever-37, T2 toxin-38, Saxitoxin-39.

List of agents	Assay shelf life	Assay cost	Analyzing capacity	Certified
1,4,16,9,20,2	24 months from date of manufacture	$257/10 pack	Single	DHS
1,4,16,9,20	24 months from date of manufacture	$735/10 pack	Multiple	DHS
1,16,2,18,4,9,20,5	18 months from date of manufacture	$995/10 pack	Multiple	–
1,16,37,2,18,4,9,20,5,33,23	24 months from date of manufacture	Complete kit—$9000	Multiple	–
1,16,2,9,20,5,34	12 months	$1270/10 pack	Multiple	ISO 9001:2008-certified
1,4,9,20	12–24 months	$400–$650/10 pack	Single	ISO 9001:2008-certified
1,16,37,2,18,4,9,19,20,32, 38,39, 7.1,7.2,7.3,35	12 months	$1–$4/assay		–
1,16,37,4,9,20	12 months	$575/25 pack	Single	–
1,16,2,18,4,9,20,5,23,36		$2000/10 pack	4-agent assay	–
1,18,4,9	12 months	$675/25 pack	Single	–
1,16,2,4,9,19,20,5	2 years	$605/25 pack	Single	–

Conclusion

The detection of biological agents is a challenging task, particularly in the outdoor environment. The developments of technologies with rapid and sensitive detection capabilities have become crucial in the present scenario of emerging bioterrorism. Researchers around the world have taken a number of different avenues in their search for a biosensor. Most of the studies report bench-top studies for proof of concept. Very few describe robust analytical results from the field. Successful implementation of a national biodefense strategy will require integration of a variety of independent efforts across the government agencies, bioscience research, and medical/public health communities.

Commercial molecular detection platforms

BioFire Defense (FilmArray)
[BioFire Diagnostics/
Biomerieux, France]

Bio-Seeq PLUS
[BioFire Diagnostics/ Biomerieux, France]

RAZOR EX
[BioFire Defense, LLC, USA]

T-COR 8
[Tetracore, USA]

POCKIT
[GeneReach Biotech Corp, Taiwan]

POCKIT Micro Nucleic Acid Analyzer
[GeneReach Biotech Corp, Taiwan]

Commercial immunological detection platforms

Pro Strips
[AdVnt Biotechnologies,LLC]

RAMP
[Response Biomedical Corp.]

BioThreat Alert
[Tetracore, Inc.]

Envi Assay
[Environics, Inc.]

RAPTOR 4 channel
[Research International, Inc.]

NIDS
[ANP Technologies Inc.]

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