

Biosensors for Security and Bioterrorism: Definitions, History, Types of Agents, New Trends and Applications

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Abstract Biosensors are making a large impact in environmental, food, biomedical, and in many other applications. They provide many advantages. in comparison to standard analytical detection methods (i.e., chromatographic techniques) such as minimal sample preparation and handling, faster time analysis, simpler steps of analysis, rapid detection of the analytes of concern, use of non-skilled personnel, and portability for uses in the field applications. The aim of this chapter is to focus on novel research related to the rapid detection of agents and weapons of bioterrorism and provide a comprehensive review of the research topics most pertinent to advancing devices applicable to the rapid real-time detection of toxicants and bioterrorism weapons such as microbes, pathogens, toxins, virus, or nerve gases. The ongoing war on terrorism and the rising security concerns are driving the need for newer faster biosensing devices against bio-warfare agents for both military and civil defense applications. Readers of these review article will learn new schemes of biological weapons that can lead to the construction of devices that will minimize the risk of bio-terrorism.

Keywords Biosensors · Bioterrorism · Virus · Bacteria · Toxins · Nerve gases

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1 Introduction

Recent events have made public health officials acutely aware of the importance to rapidly and accurately detect bioterrorism. Because bioterrorism is difficult to predict or prevent, reliable platforms to rapidly detect and identify bioterrorism agents are important in order to minimize the spread and wide use of these agents and to protect the public health. These platforms must be sensitive, specific, and must also be able to accurately detect a variety of pathogens, including modified or previously uncharacterized agents, directly from complex sample matrices. Recent developments in laboratory prototype devices have been evaluated and were commercialized. Various commercial tests that utilize biochemical, immunological, nucleic acid, and bioluminescence procedures are currently available to identify biological threat agents. Recent developed tests identify bioterrorism agents using DNA aptamers, biochips, evanescent wave biosensors, cantilevers, living cells, and other innovative technologies. This review describes current and developing technologies against bioterrorism and considers challenges to rapid, accurate detect biothreat agents. Although there is no ideal platform, many of these technologies have proved valuable for the detection and identification of bioterrorism agents.

Various tests have been developed to detect and identify biothreat agents. Some of these tests were available before 11 September 2001; other tests have been developed since that time. Although many of these technologies claim to be rapid, accurate, and reliable, few have been evaluated under field conditions for portable uses by non-skilled personnel. This review describes documented current and developing technologies for detection and identification of bioterrorism weapons and addresses the challenges associated with detection in complex sample matrices.

2 Definitions

A bioterrorism attack is the deliberate release of viruses, bacteria, toxins or other harmful agents used to cause illness or death in people, animals, or plants. These agents are typically found in nature, but it is possible that they could be mutated or altered to increase their ability to cause disease, make them resistant to current medicines, or to increase their ability to be spread around the environment. Biological agents can be spread through the air, water, or in food. Terrorists tend to use biological agents because they are extremely difficult to detect and do not cause illness for several hours to several days. Some bioterrorism agents, like the smallpox virus, can be spread from person to person and some, like anthrax, cannot.

Bioterrorism is an attractive weapon because the toxicants are relatively easy and inexpensive to obtain, can be easily disseminated, and can cause widespread fear and panic beyond the actual physical damage. Military leaders, however, have learned that, as a military asset, bioterrorism has some important limitations; it is

difficult to employ a biological weapon in a way that only the enemy is affected and not friendly forces. A biological weapon is useful to terrorists mainly as a method of creating mass panic and disruption to a state or a country. However, technologists have warned of the potential power which genetic engineering might place in the hands of future bio-terrorists.

3 History

There have been many reviews written regarding the history, theory, and use of bioterrorism, biothreats, biological weapons, and biological warfare from the 14th century to today. The reader is referred to the following for additional information: Atlas [1], Christopher et al. [2], Hawley and Eitzen [3], Heden [4], Klietmann and Ruoff [5], van Courtland Moon [6], and Tucker [7].

The era of biological weapons was significantly advanced in the 20th century by modern microbiology and multiple international wars. The biological and chemical horrors inflicted during World War I resulted in the drafting of the 1925 Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases and of Bacteriological Methods of Warfare. However, many countries that signed the document did so with contingencies in the event of attack by a non-ratifying entity and with the stipulation that the protocol did not prevent investigative research.

Subsequently, Germany, Japan, the Union of Soviet Socialist Republics, and the United States initiated research programs to eliminate the threats of biological weapons [2]. In 1969, under President Richard M. Nixon, the United States began dismantling its offensive biological weapon programs. Henceforth, all biothreat agent research programs in the United States were of a defensive nature, and the 1972 Geneva Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological and Toxin Weapons and on Their Destruction was developed and ratified. However, several countries that signed the convention (notably the Union of Soviet Socialist Republics and Iraq) continued offensive research and production of biological agents as recently as the mid-1990s. Additionally, there have been increasingly more subnational terrorist and radical groups that have independently worked on offensive use of biological weapons since the mid-1980s to today [7].

3.1 20th Century

By the time World War I began, attempts to use anthrax were directed at animal populations. Shortly after the start of World War I, Germany launched a biological sabotage campaign in the United States, Russia, Romania, and France. Anton

Dilger was sent to the United States in 1915 carrying cultures of glanders, a virulent disease of horses and mules. Dilger set up a laboratory in his house. He used stevedores working in the docks in Baltimore to infect horses with glanders while they were waiting to be shipped to Britain. Dilger was under suspicion as being a German agent, but was never arrested. Dilger eventually fled to Madrid, Spain, where he died during the Influenza Pandemic of 1918. In 1916, the Russians arrested a German agent with similar intentions. Germany and its allies infected French cavalry horses and many of Russia's mules and horses on the Eastern Front. These actions hindered artillery and troop movements, as well as supply convoys.

In Oregon in 1984, followers of the Bhagwan Shree Rajneesh attempted to control a local election by incapacitating the local population. This was done by infecting salad bars in 11 restaurants, produced in grocery stores, doorknobs, and other public domains with *Salmonella typhimurium* bacteria in the city of The Dalles, Oregon. The attack infected 751 people with severe food poisoning. There were no fatalities. This incident was the first known bioterrorist attack in the United States in the 20th century.

In June 1993 the religious group Aum Shinrikyo released anthrax in Tokyo. Eyewitnesses reported a foul odor. The attack was a total failure, infecting not a single person. The reason for this, ironically, is that the group used the vaccine strain of the bacterium. The spores recovered from the attack showed that they were identical to an anthrax vaccine strain given to animals at the time. These vaccine strains are missing the genes that cause a symptomatic response.

3.2 21st Century

2001—USA and Chile—Anthrax Attacks: In September and October 2001, several cases of anthrax broke out in the United States in the 2001 anthrax attacks, apparently caused deliberately. Letters laced with infectious anthrax were concurrently delivered to news media offices and the U.S Congress, alongside an ambiguously related case in Chile. The letters killed 5 people.

4 Types of Agents

4.1 Category A

These high-priority agents pose a risk to national security, can be easily transmitted and disseminated, result in high mortality, have potential major public health impact, may cause public panic, or require special action for public health preparedness.

4.1.1 Anthrax

Anthrax is a non-contagious disease caused by the spore-forming bacterium *Bacillus anthracis*. An anthrax vaccine does exist but requires many injections for stable use. When discovered early anthrax can be cured by administering antibiotics (such as ciprofloxacin).

4.1.2 Smallpox

Smallpox is a highly contagious virus. It is transmitted easily through the atmosphere and has a high mortality rate (20-40 %). Smallpox was eradicated in the world in the 1970s, thanks to a worldwide vaccination program. However, some virus samples are still available in Russian and American laboratories. Some believe that after the collapse of the Soviet Union, cultures of smallpox have become available in other countries. Although people born pre-1970 would have been vaccinated for smallpox under the WHO program, the effectiveness of vaccination is limited since the vaccine provides high level of immunity for only 3 to 5 years. Revaccination's protection lasts longer. As a biological weapon smallpox is dangerous because of the highly contagious nature of both the infected and their pox. Also, the infrequency with which vaccines are administered among the general population since the eradication of the disease would leave most people unprotected in the event of an outbreak. Smallpox occurs only in humans, and has no external hosts or vectors.

4.1.3 Botulinum Toxin

Botulinum toxin is one of the deadliest toxins known, and is produced by the bacterium *Clostridium botulinum*. Botulism causes death by respiratory failure and paralysis. Furthermore, the toxin is readily available worldwide due to its cosmetic applications in injections.

4.1.4 Bubonic Plague

Plague is a disease caused by the *Yersinia pestis* bacterium. Rodents are the normal host of plague, and the disease is transmitted to humans by flea bites and occasionally by aerosol in the form of pneumonic plague. The disease has a history of use in biological warfare dating back many centuries, and is considered a threat due to its ease of culture and ability to remain in circulation among local rodents for a long period of time. The weaponized threat comes mainly in the form of pneumonic plague (infection by inhalation).

4.1.5 Viral Hemorrhagic Fevers

This includes hemorrhagic fevers caused by the Filoviridae (Marburg and Ebola), and by the Arenaviridae (for example the Lassa fever and the Bolivian hemorrhagic fever). Ebola has fatality rates ranging from 50-90 %. No cure currently exists, although vaccines are in development. Death from Ebola is commonly due to multiple organ failure and hypovolemic shock. Marburg was first discovered in Marburg, Germany. No treatments currently exist aside from supportive care. The arenaviruses have a greatly reduced fatality rate, but a larger presence, chiefly in central Africa and South America.

4.1.6 Tularemia

Tularemia, or rabbit fever, has a very low fatality rate if treated, but can severely incapacitate. The disease is caused by the *Francisella tularensis* bacterium, and can be contracted through contact with the fur, inhalation, or ingestion of contaminated water or insect bites.

4.2 Category B

Category B agents are moderately easy to disseminate and have low mortality rates.

- Brucellosis (*Brucella* species)
- Epsilon toxin of *Clostridium perfringens*
- Food safety threats (e.g., *Salmonella* species, *E. coli* O157:H7, *Staphylococcus aureus*)
- Psittacosis
- Q fever
- Staphylococcal enterotoxin B
- Typhus (*Rickettsia prowazekii*)
- Viral encephalitis
- Water supply threats (e.g., *Cryptosporidium parvum*)

4.3 Category C

Category C agents are emerging pathogens that might be engineered for mass dissemination because of availability, easy to produce and disseminate, or may possess high mortality or a major health impact.

Typical category C agents are;

- Nipah virus
- Hantavirus
- SARS
- H1N1 a strain of influenza (flu)
- HIV/AIDS

5 Recent Advances of Biosensors for the Rapid Detection of Weapons of Terrorism

Recent biological terrorism threats and outbreaks of microbial pathogens clearly emphasize the need for biosensors that can quickly and accurately identify infectious agents. The majority of rapid biosensors generate detectable signals when a molecular probe in the detector interacts with an analyte of interest. Analytes may be whole bacterial or fungal cells, virus particles, or specific molecules, such as chemicals or protein toxins, produced by the infectious agent. Peptides and nucleic acids are most commonly used as probes in biosensors because of their versatility in forming various tertiary structures. The interaction between the probe and the analyte can be detected by various sensor platforms, including quartz crystal microbalances, surface acoustical waves, surface plasmon resonance, amperometrics, and magnetoelastics. The field of biosensors is constantly evolving to develop devices that have higher sensitivity and specificity, and are smaller, portable, and cost-effective. The present article describes recent advances in biosensors for applications in the rapid detection of bioterrorism weapons.

Although biosensors are exhibiting double-digit growth rates, they still have to overcome a number of challenges, including the following:

- New research focus less into fundamental research due to impeding introduction of newer applications
- Development of a single biosensor platform with multi-purpose diagnostics capability has restricted biosensor applications
- Numerous problems encountered in successful commercialization of biosensors have encouraged conservative development strategies
- Competition from non-biosensor technologies has hindered revenue growths
- Low rate of technology transfer and lower level of development has deterred the development newer biosensors

Given the current zeitgeist, the market thrust has shifted to biosensors' security capabilities amid the hot topic of biowarfare. A recent report from market research firm In-Stat revealed that the media spotlight on this application may be premature: Despite the public's anticipation that biosensors with real-time detection will be

able to monitor biological and chemical weapons, the technology hasn't caught up with expectations. Presently, biosensors in environmental monitoring stations nationwide can detect compounds like anthrax—but detection can take 12–24 h.

6 Planning and Response

Planning may involve the development of biological identification systems. Until recently, most biological defense strategies have been geared to protecting soldiers on the battlefield rather than ordinary people in cities. Financial cutbacks have limited the tracking of disease outbreaks. Some outbreaks, such as food poisoning due to *E. coli* or *Salmonella*, could be of either natural or deliberate origin.

6.1 Preparedness

Biological agents are relatively easy to obtain by terrorists and are becoming more threatening, and laboratories are working on advanced detection systems to provide early warning, identify contaminated areas and populations at risk, and to facilitate prompt treatment. Methods for predicting the use of biological agents in urban areas as well as assessing the area for the hazards associated with a biological attack are being established in major cities. In addition, forensic technologies are working on identifying biological agents, their geographical origins and/or their initial source. Efforts include decontamination technologies to restore facilities without causing additional environmental concerns.

Early detection and rapid response to bioterrorism depend on close cooperation between public health authorities and law enforcement; however, such cooperation is currently lacking. National detection assets and vaccine stockpiles are not useful if local and state officials do not have access to them.

6.2 Aspects of Protection Against Bioterrorism Mainly in the US Include

- **Detection and resilience strategies in combating bioterrorism.** This occurs primarily through the efforts of the Office of Health Affairs (OHA), a part of the Department of Homeland Security (DHS), whose role is to prepare for an emergency situation that impacts the health of the American populace. Detection has two primary technological factors. First there is OHA's BioWatch program in which collection devices are disseminated to thirty high risk areas throughout the country to detect the presence of aerosolized biological agents before

symptoms present in patients [8]. This is significant primarily because it allows a more proactive response to a disease outbreak rather than the more passive treatment of the past.

- **Implementation of the Generation-3 automated detection system.** This advancement is significant simply because it enables action to be taken in four to six hours due to its automatic response system, whereas the previous system required aerosol detectors to be manually transported to laboratories [8]. Resilience is a multifaceted issue as well, as addressed by OHA. One way in which this is ensured is through exercises that establish preparedness; programs like the Anthrax Response Exercise Series exist to ensure that, regardless of the incident, all emergency personnel will be aware of the role they must fill. Moreover, by providing information and education to public leaders, emergency medical services and all employees of the DHS, OHS suggests that it can significantly decrease the impact of bioterrorism [8].
- **Enhancing the technological capabilities of first responders.** This is accomplished through numerous strategies. The first of these strategies was developed by the Science and Technology Directorate (S&T) of DHS to ensure that the danger of suspicious powders could be effectively assessed, (as many dangerous biological agents such as anthrax exist as a white powder). By testing the accuracy and specificity of commercially available systems used by first responders, the hope is that all biologically harmful powders can be rendered ineffective.
- **Enhanced equipment for first responders.** One recent advancement is the commercialization of a new form of Tyvex™ armor which protects the first responders and patients from chemical and biological contaminants. There has also been a new generation of Self-Contained Breathing Apparatuses (SCBA) which has been recently made more robust against bioterrorism agents. All of these technologies combine to form what seems like a relatively strong deterrent to bioterrorism. However, New York City as an entity has numerous organizations and strategies that effectively serve to deter and respond to bioterrorism as it comes. From here the logical progression is into the realm of New York City's specific strategies to prevent bioterrorism.
- **BioShield** The accrual of vaccines and treatments for potential biological threats, also known as medical countermeasures has been an important aspect in preparing for a potential bioterrorist attack; this took the form of a program beginning in 2004, referred to as Project BioShield. The significance of this program should not be overlooked as there is currently enough smallpox vaccine to inoculate every United States citizen and a variety of therapeutic drugs to treat the infected. The Department of Defense also has a variety of laboratories currently working to increase the quantity and efficacy of countermeasures that comprise the national stockpile [9]. Efforts have also been taken to ensure that these medical countermeasures are able to be disseminated effectively in the event of a bioterrorist attack. The National Association of Chain Drug Stores championed this cause by encouraging the participation of the private sector in improving distribution of such countermeasures if required [9].

On a CNN news broadcast in 2011, the CNN chief medical correspondent, Dr. Sanjay Gupta, weighed in on the American government's recent approach to bioterrorist threats. He explains how, even though the United States would be better fending off bioterrorist attacks now than they would be a decade ago, the amount of money available to fight bioterrorism over the last three years has begun to decrease. Looking at a detailed report that examined the funding decrease for bioterrorism in fifty-one American cities, Dr. Gupta stated that the cities "wouldn't be able to distribute vaccines as well" and "wouldn't be able to track viruses". He went on to say that movie portrayals of global pandemics, such as *Contagion*, were actually quite possible and may occur in the United States under the right conditions.

A news broadcast by MSNBC in 2010 also stressed the low levels of bioterrorism preparedness in the United States. The broadcast stated that a bipartisan report gave the Obama administration a failing grade for its efforts to respond to a bioterrorist attack. The news broadcast invited the former New York City police commissioner, Howard Safir, to explain how the government would fare in combating such an attack. He said how "biological and chemical weapons are probable and relatively easy to disperse". Furthermore, Safir thought that efficiency in bioterrorism preparedness is not necessarily a question of money, but is instead dependent on putting resources in the right places. The broadcast suggested that the nation was not ready for something more serious.

6.3 *Biosurveillance*

In 1999, the University of Pittsburgh's Center for Biomedical Informatics deployed the first automated bioterrorism detection system, called RODS (Real-Time Outbreak Disease Surveillance). RODS is designed to collect data from many data sources and use them to perform signal detection, that is, to detect a possible bioterrorism event at the earliest possible moment. RODS, and other systems like it, collect data from sources including clinic data, laboratory data, and data from over-the-counter drug sales [10]. In 2000, Michael Wagner, the Codirector of the RODS laboratory, conceived the idea of obtaining live data feeds from "non-traditional" (non-health-care) data sources. The RODS laboratory's first efforts eventually led to the establishment of the National Retail Data Monitor, a system which collects data from 20,000 retail locations nationwide [10].

The principles and practices of biosurveillance, a new interdisciplinary science, were defined and described in the *Handbook of Biosurveillance*, edited by Michael Wagner, Andrew Moore and Ron Aryel, and published in 2006. Biosurveillance is the science of real-time disease outbreak detection. Its principles apply to both natural and man-made epidemics (bioterrorism).

Data which potentially could assist in early detection of a bioterrorism event include many categories of information. Health-related data such as that from hospital computer systems, clinical laboratories, electronic health record systems,

medical examiner record-keeping systems, 911 call center computers, and veterinary medical record systems could be of help; researchers are also considering the utility of data generated by ranching and feedlot operations, food processors, drinking water systems, school attendance recording, and physiologic monitors, among others. Intuitively, one would expect systems which collect more than one type of data to be more useful than systems which collect only one type of information (such as single-purpose laboratory or 911 call-center based systems), and be less prone to false alarms, and this appears to be the case.

In Europe, disease surveillance is beginning to be organized on the continent-wide scale needed to track a biological emergency. The system not only monitors infected persons, but attempts to discern the origin of the outbreak.

Researchers are experimenting with devices to detect the existence of a threat:

- Tiny electronic chips that would contain living nerve cells to warn of the presence of bacterial toxins (identification of broad range toxins)
- Fiber-optic tubes lined with antibodies coupled to light-emitting molecules (identification of specific pathogens, such as anthrax, botulinum, ricin)

New research shows that ultraviolet avalanche photodiodes offer a high gain, reliability and robustness needed to detect anthrax and other bioterrorism agents in the air. The fabrication methods and device characteristics were described at the 50th Electronic Materials Conference in Santa Barbara on June 25, 2008. Details of the photodiodes were also published in the February 14, 2008 issue of the journal *Electronics Letters* and the November 2007 issue of the journal *IEEE Photonics Technology Letters* [11].

6.4 Response to Bioterrorism Incident or Threat

Government agencies which would be called on to respond to a bioterrorism incident would include law enforcement, hazardous materials/decontamination units and emergency medical units, if they exist.

The US military has specialized units, which can respond to a bioterrorism event; among them are the United States Marine Corps' Chemical Biological Incident Response Force and the U.S. Army's 20th Support Command (CBRNE), which can detect, identify, and neutralize threats, and decontaminate victims exposed to bioterror agents. US response would include the Center for Disease Control.

Historically, governments and authorities have relied on quarantines to protect their populations. International bodies such as the World Health Organization already devoted some of their resources to monitoring epidemics and have served clearing-house roles in historical epidemics.

Media attention toward the seriousness of biological attacks increased in 2013-2014. In July 2013, *Forbes* published an article with the title "Bioterrorism: A Dirty Little Threat With Huge Potential Consequences" [12]. In November 2013,

Fox News reported on a new strain of botulism, saying that the Centers for Disease and Control lists botulism as one of two agents that have “the highest risks of mortality and morbidity”, noting that there is no antidote for botulism [13]. USA Today reported that the U.S. military in November was trying to develop a vaccine to protect troops from the bacteria that cause the disease Q fever, an agent the military once used as a biological weapon.

7 Conclusions

This review has attempted to provide a survey of commercially available and developing technologies for biothreat agent detection. Only technologies that have been evaluated and published have been included. Many other technologies have not been included because insufficient published data were available to ascertain their accuracy and reliability. While an ideal platform has yet to be developed, many of the systems described in this review have proved invaluable in rapidly and accurately identifying biothreat agents. Although the risk of bioterrorism remains, detection technologies will continue to be improved to meet the challenges of this threat.

Security and biodefense is emerging as a strong market for new applications. The new biosensor technology has significant technological advantages when compared to that of the traditional detection methods; for example, vesicles for use in biosensors have both high specificity and high sensitivity (where the vesicles include a receptor specific for the intended analyte and a signal generating component).

We are looking into portable and handheld biosensors, for example, such as dynamic DNA and Protein Arrays for rapid and accurate detection of pathogens. Bioweapons are extremely damaging and efficient. Progress of biotechnology opens also new routes for weaponizing pathogens.

Challenges for biothreat detection are:

1. High sensitive—detect very small amounts of pathogens, toxins, and chemical agents.
2. Highly selective—discriminate targets from other materials
3. Massively parallel to detect multiple pathogens, minimize false positive, have rapid response, without sample preparation
4. Transportable or handheld, robust, simple to operate
5. Inexpensive
6. Adaptable to new biothreats, integrated chemical-biosensor
7. and finally allow the detection of single molecules.

Targets to achieve are:

1. Single RNA molecule detection.
2. Real-time monitoring of RNA hybridization at single molecule level.

3. Real-time monitoring of protein binding to aptamers at single molecule level.
4. Hybridization of synthetic target DNA with anti-anthrax.
5. Selectivity of protein detection, i.e., selectivity of human thrombin detection by anti-thrombin aptamers.

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