

Chemical and Biological Weapons: Current Concepts for Future Defenses

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In the post-9/11 era, the asymmetric threats posed by terrorists or rogue states have created new challenges for the enhanced and efficient defense of the nation. For defense against chemical and biological weapons (CBW), integrated, multitiered, and “net-centric” systems are envisioned that will enable the rapid and cost-effective detection, confirmation, and response to a CBW attack. Realization of this vision requires advances in the science and technology of chemical and biological sensor systems and multisource information fusion. Our evolving counter-CBW capability has broader benefits to society, where, for example, new tools will become available to manage outbreaks of emerging natural infectious diseases or industrial accidents. Here we highlight several key technologies and the challenges pursued in support of this vision.

INTRODUCTION

The changing reality of asymmetric threats facing the nation in the 21st century is best reflected by the September 11 attacks and the subsequent distribution through the U.S. postal service of anthrax-spore-laced letters. These events, as well as earlier occurrences (e.g., the Aum Shinrikyo attacks in Tokyo in 1995), highlight the need for rapid development of effective and efficient approaches to defending military and large civilian populations against current and emerging chemical/biological weapons (CBW) (Fig. 1). The threat of CBW use, both at home and abroad by individual terrorist groups or rogue states, has not diminished despite international efforts to control agent proliferation. For BW in particular, it is predicted that both their proliferation and the likelihood for eventual use will increase significantly over the next decades.^{1,2} Several presidential

directives^{3–6} spell out in further detail the National Strategy to Combat Weapons of Mass Destruction (2002)⁷ in the area of countering CBW threats.

Many of the current methods for the production and dispersal of CBW are based on well-established, inexpensive, and accessible technology from the 1950s. In contrast, adequate responses to these threats require the most advanced scientific and technological achievements in disciplines as diverse as supercomputer modeling of atmospheric processes to molecular biology.^{8–10} New interdisciplinary approaches, integrating traditional scientific disciplines, are being developed for improving responses to CBW threats. For example, the emerging field of microbial forensics¹¹ uses various analytical methods to reverse-engineer the processes and conditions of growth of pathogenic agents as a tool

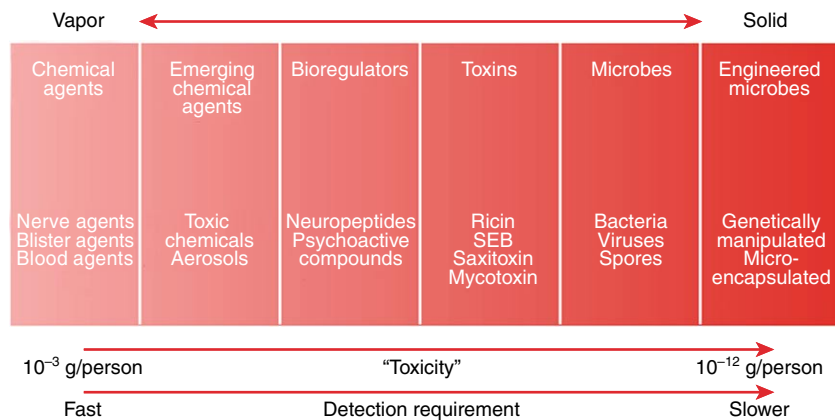


Figure 1. CBW threats vary greatly in physical properties and mechanisms of physiological action (SEB = staphylococcal enterotoxin B).

for intelligence and attribution. Although increasingly more sophisticated systems for detection and identification of CBW are being developed, the need is obvious for systems with much higher sensitivity and specificity, greater automation, reduced cost, and potential for field deployment.^{8,12} Unfortunately, recent advances in biological science, such as genetic engineering, potentially can result in the development of far more potent weapons, while defenses against them would become even more difficult. To quote from a recently released National Intelligence Council report:

Over the next 10 to 20 years there is a risk that advances in biotechnology will augment not only defensive measures but also offensive biological warfare agent development and allow the creation of advanced biological agents designed to target specific systems—human, animal, or crop.¹

This possibility has even raised issues associated with the free dissemination and publication of scientific data. Systems being deployed to counter such asymmetric threats are still in their early developmental evolution and require an investment in science and technology across a broad range of disciplines. It should be stressed, however, that CBW defenses are a “dual-use” technology as well. For instance, our improved capabilities to fight the deliberate uses of bioterror agents, causing, say, anthrax, plague, or smallpox, will also dramatically improve our response to outbreaks of emerging natural infectious diseases (e.g., SARS, bird flu viruses).

The pillars of the national biodefense program have been identified as³⁻⁶

- Threat awareness
- Prevention and protection
- Surveillance and detection
- Post-attack response and recovery

A primary goal for an effective CBW defense system, given that prevention may not be 100% effective, is to provide a timely response to an attack, including a number of countermeasures. Many information sources

must be analyzed and integrated to enable a timely and appropriate response (Fig. 2). The cost-effectiveness of various components of such a system is still hotly debated.^{13,14} The relative contribution of each component for threat assessment and ultimate protection will obviously depend on the specific conditions, i.e., local (e.g., facility or vessel) versus global (city or region) defenses. Likewise, the requirements and selection for deployment of any such system would vary depending on the final user—be it a first responder on the homeland security front or a soldier on the battlefield—and the most likely scenario for CBW deployment.

SCIENCE AND TECHNOLOGY CHALLENGES IN CHEMICAL AND BIOLOGICAL WEAPONS DEFENSE

For several decades, APL has been pursuing advances in science and technology to address critical challenges in the development of reliable, affordable, and effective defenses against CBW attacks. These challenges comprise a subset of the challenges facing the nation for which APL is positioned to make significant contributions. Challenges that are outside the APL mission, such as rapid drug and vaccine development, are not discussed here.

Underlying a robust surveillance and detection regime are the various sensor systems used to detect the presence of a chemical or biological warfare agent. A major challenge for any CBW sensor is the *uniqueness* of the signature (*specificity* of the response) produced by the sensor. This response is based on the precise physical and/or chemical properties of the targeted agent. Depending

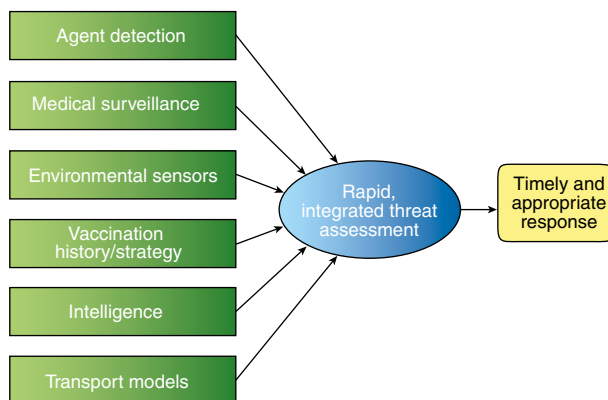


Figure 2. Diverse information sources need to be integrated to provide a rapid, adequate, and relevant response to a CBW attack.

on the particular target, sensor response uniqueness can vary widely. For CW agents, toxic industrial chemicals, and biological toxins, the detection target is usually an agent-specific molecule or a small set of molecules. For BW, there are a variety of potential molecular targets—DNA, RNA, proteins, metabolites—that allow identification of a particular microorganism. Since DNA sequence information is available only for a fraction of the microbial “universe,” the uniqueness of target-organism sequences used for DNA-based detection can only be assessed with respect to the available genome sequence data. Thus, rapid identification of an emergent or bioengineered threat (with unknown DNA sequence) represents a significant technological challenge. Such a challenge could be met by future sensors that employ rapid whole genome sequencing.

The rapid identification of CBW outside the laboratory poses another daunting challenge, frequently likened to the proverbial “needle in a haystack” problem: the agents in trace amounts must be detected in *complex backgrounds* (soil, seawater, bodily fluids, etc.). These backgrounds contain chemicals that may inhibit the sensor and/or “clutter” that can confound agent detection. Real-world backgrounds are diverse and highly variable and can impact sensor performance unpredictably (e.g., limit of detection). How then does one objectively characterize the performance of a biological sensor when the admixture of potential confounders in any given sample is not known?

Receiver-operator-characteristics (ROC) curves allow the characterization of sensor performance trade-offs: probability of detection (related to sensitivity) versus probability of false alarm (related to specificity) at varying detection thresholds. ROC curves are, however, only meaningful for the specified background and specified concentration of the target in that background. Additional rigorous mathematical representations are clearly needed to characterize performance more generally. A strategy to mitigate the effects of varying backgrounds is to isolate the CBW agents (target molecules) from backgrounds by sample preparation. The development of rapid, automated sample preparation schemes has thus become a critical technology challenge. The trade-offs among analysis speed, ease of use (e.g., automation of sample preparation devices), and the sensitivity and specificity of different biosensors in discrete operating environments necessitate a “tiered sensor suite approach” to both chemical and biological threat assessment: a large number of rapid, easy-to-use sensors on the front lines, followed by more labor-intensive, time-consuming confirmatory detection. As confirmatory-type assays become more field portable, new approaches for assigning confidence values to a threat assessment from an integrated biosensor suite will need to be developed.

Another challenge is further development of computational approaches for *quantitative structure–activity*

relationships to determine the chemical toxicity of the molecular structure or the virulence of a pathogen from DNA gene sequence information. For instance, such informatics approaches would allow us to infer toxicity for a detected chemical from its structural similarity to other known toxic chemicals when no human toxicological data for that chemical are available. Both short- and long-term toxicity effects of acute or low-level exposures need to be predicted by such approaches. While this problem goes beyond sensor technology, meeting the challenge will have a significant impact on determining the appropriate response to a CBW attack or an industrial incident.

A further challenge for BW defense is posed by the *requirements to defend large populations* (large areas). In most such scenarios, the likelihood of directly detecting a BW surreptitiously released into the atmosphere is exceedingly low because of the low spatial coverage of the biosensors. In this case, human “sentinels” represent the frontline systems for detecting the release of a human pathogen. Here, medical surveillance and surveillance of nontraditional indicators such as sales of over-the-counter drugs (“syndromic surveillance”) can be used to detect early indications of a BW-induced disease outbreak. The sheer volume, noisiness, and inherent variation of nontraditional surveillance data present a daunting data-mining obstacle. However, intelligent information fusion incorporating epidemiological knowledge has the potential to meet this challenge. A significant technical hurdle is to encode the delicate interplay between expert knowledge and real-time data feeds into automated algorithms for alerting with acceptable false alarm rates. Finally, as such surveillance-based systems evolve to additionally fuse available sensor and intelligence data, a truly net-centric biodefense capability will emerge.

The development of cost-effective neutralization strategies for intercepting a released cloud of CBW agent during an attack, as well as decontamination after an attack, also presents difficult challenges. One potential use of CBW agents is to deny the availability of critical infrastructure or assets (e.g., the Pentagon or Wall Street). For small or well-controlled environments, reasonable solutions have been effectively deployed. The challenge is in rapidly protecting or verifiably decontaminating large, uncontrolled environments without introducing new hazards to human health and/or destroying valuable assets in the process.

In the following sections, we highlight some current achievements, developments in methodologies, and promising future concepts in light of the science and technology challenges presented above. We focus on two specific elements of a system for defenses against CBW: novel sensor technologies for agent detection and information fusion for rapid integrated threat assessment. The emphasis here is on defenses against BW, given

their projected higher lethality among civilian populations.^{15,16} We briefly discuss several types of sensors and technologies currently being developed at APL and elsewhere for detection of a CBW attack. A comprehensive review on CBW sensor technology is beyond the scope of this article. We refer the reader to several publications that discuss in more detail other promising techniques not included here.^{8,17–25}

THE NATURE OF THE CHEMICAL/BIOLOGICAL WEAPONS THREAT

Chemical Weapons

These weapons are either synthetic or biologically derived (natural) chemical compounds that are lethal in doses of much less than 1 g per person when inhaled, ingested, injected, etc. (Fig. 1). The modes of action of CW are very rapid and require immediate response—almost instantaneous detection, individual protection, treatment, and decontamination.¹⁶ The homogeneous nature of CW agents makes the development of sensor systems simpler than for BW agents. Compared to BW, however, CW possess lower lethality; it is estimated that 100 kg of anthrax spore powder, released on a clear and calm night, can affect an area of about 300 km², which projected over greater Washington could result in more than 1 million deaths. In contrast, the release of a 10-times larger quantity, say, 1000 kg of sarin gas, would affect an area less than 8 km², resulting in about 3000 deaths.¹⁵ The task of detecting a CW agent is also simpler than for a BW agent, given the relative simplicity of a homogeneous chemical composition, allowing the physical properties of the agent to be exploited for rapid detection and discrimination from background materials. On the other hand, detection of low vapor pressure chemicals (e.g., CW precursors) still presents technological challenges in the field. In addition, while not specifically characterized as warfare agents, many toxic industrial chemicals could be used by terrorists as weapons of economic disruption and to wreak havoc on a population.

Biological Weapons

It has been recognized that more than 1400 infectious organisms, among them more than 200 viral and 500 bacterial species, can be pathogenic to humans.²⁶ All toxins and microorganisms (live viruses, bacterial spores, vegetative bacterial cells, etc.) currently considered a threat to the nation are classified by the Centers for Disease Control (see the boxed insert) into three tiered categories (A, B, C). Agents belonging to category A are considered to be the most dangerous and the easiest to convert into potent BWs. The second highest priority agents are in category B and include those that are moderately easy to disseminate, result in moderate morbidity rates and low mortality rates, and require

CDC LIST OF SELECTED DISEASES AND AGENTS

Category A

The U.S. public health system and primary health care providers must be prepared to address various biological agents, including pathogens that are rarely seen in the United States. These highest-priority agents include organisms that pose a risk to national security because they can be easily disseminated or transmitted from person to person, result in high mortality rates, have the potential for major public health impact, might cause public panic and social disruption, and require special action for public health preparedness.

Disease/agent

- Anthrax (*Bacillus anthracis*)
- Botulism (*Clostridium botulinum* toxin)
- Plague (*Yersinia pestis*)
- Smallpox (variola major)
- Tularemia (*Francisella tularensis*)
- Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo])

Category B

These second highest priority agents include those that are moderately easy to disseminate, result in moderate morbidity rates and low mortality rates, and require specific enhancements of CDC's diagnostic capacity and disease surveillance.

Disease/agent

- Brucellosis (*Brucella* species)
- Enterotoxemia (*Clostridium perfringens* epsilon toxins)
- Foodborne diseases (e.g., *Salmonella* species, *Escherichia coli* O157:H7, *Shigella*)
- Glanders (*Burkholderia mallei*)
- Melioidosis (*Burkholderia pseudomallei*)
- Psittacosis (*Chlamydia psittaci*)
- Q fever (*Coxiella burnetii*)
- Ricin poisoning (*Ricinus communis* [castor bean plant])
- Staphylococcal enterotoxin B poisoning (*Staphylococcus aureus*)
- Typhus fever (*Rickettsia prowazekii*)
- Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])
- Waterborne diseases (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)

Category C

The third highest priority agents include emerging pathogens that could be engineered for mass dissemination in the future because of availability, ease of production and dissemination, and potential for high morbidity and mortality rates and major health impact. Examples of diseases caused by these emerging infectious agents are Nipah virus and hantavirus.

Source: <http://www.bt.cdc.gov/agent/agentlist-category.asp>. Additional data on potential chemical and biological agents can be found at the website of the Chemical and Biological Defense Information Analysis Center (<http://www.cbiac.apgea.army.mil>).

specific enhancements of CDC’s diagnostic capacity and disease surveillance. The third highest priority agents in category C include emerging pathogens that could be engineered for mass dissemination in the future because of availability, ease of production and dissemination, and potential for high morbidity and mortality rates and major health impact.

A biological attack is more lethal the longer it remains undetected (particularly if the agent is transmitted directly from person to person). By the time obvious manifestations of a disease can lead to unambiguous diagnosis by medical professionals, quarantine, prevention, and treatment options are severely limited. Loss of life can be minimized if a BW attack is detected early; however, early detection is particularly difficult for BW agents, since most are biological organisms. Pathogenic organisms contain essentially the same chemical and biochemical compounds as nonpathogenic organisms, requiring sophisticated analysis to differentiate the two groups at the molecular level. In addition, early symptoms of an infection caused by a BW agent are typically nonspecific (e.g., resembling the common flu), as the human body musters the same defenses against various invading microorganisms. When the symptoms become more specific to a BW agent, the effectiveness of medical treatment is much diminished.

DEFENSE STRATEGIES

The most effective strategy for CBW defense in response to an attack is driven by the attack timescale (Fig. 3a). The characteristic times to react to a CBW

attack can be categorized as immediate, mid-term, and long-term (Fig. 3b). An immediate detection and response (seconds to minutes following an attack) promises to save the most lives by either destroying the agent before anyone is exposed or detecting it so quickly that protective measures can be taken. As a result of the rapid effects of CW agents, immediate response is the primary life-saving response to a CW attack. A mid-term detection and response (hours to days following an attack) will still greatly reduce the loss of life by allowing timely treatment and prevention of additional exposure. A long-term detection and response (days to weeks following an attack) will not, on its own, adequately protect people, but is required for forensic analysis and remediation/decontamination of the area that was attacked. An effective response to CBW attack, either immediate or mid-term, requires that decision makers have an appropriate “situational awareness,” which in turn requires the integration of information from the disparate sources providing CBW detection.

Sensor systems needed to detect CBW attacks have a number of requirements based on the operational environment. A useful representation of the parameter space, spanning competing requirements for an individual sensor, is the spider chart²⁷ shown in Fig. 4. The ideal BW sensor (i.e., having negligible acquisition and operating costs, sensitivity to one organism, no false alarms) does not exist. An efficient strategy is to deploy a large number of inexpensive but nonspecific triggers hierarchically layered with fewer, more expensive presumptive detection devices, which are followed by sophisticated confirmatory identification sensors systems.

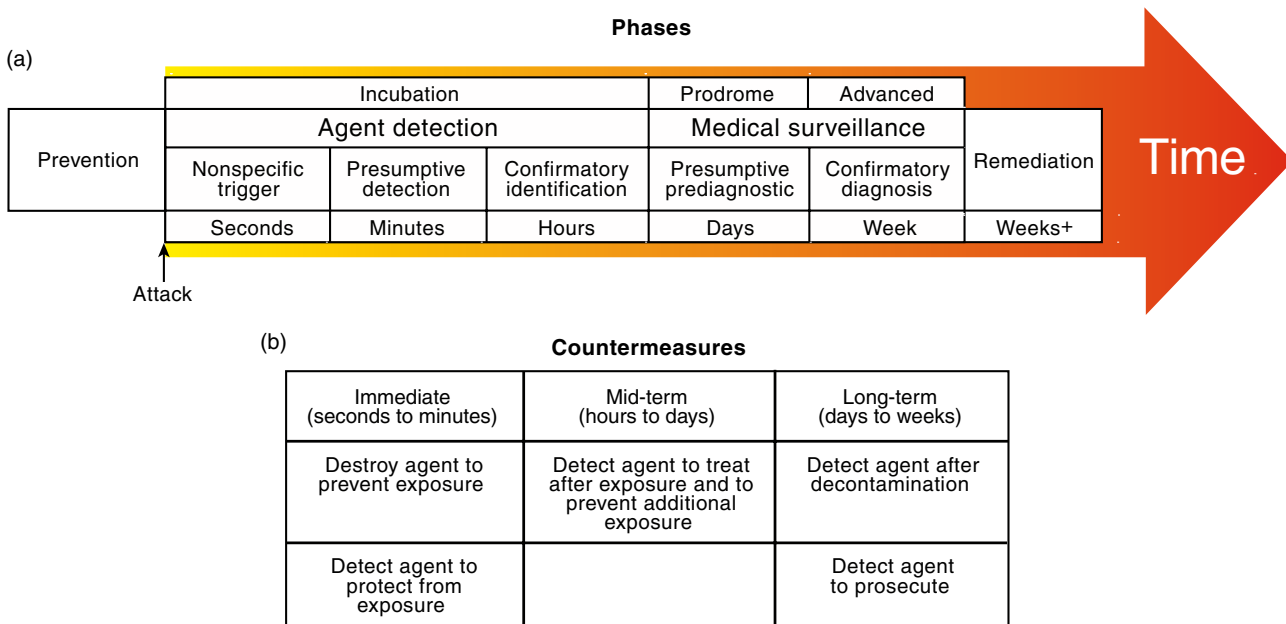


Figure 3. Attack timescale. (a) Approximate timeline of different stages to detect and mitigate a biological attack, with risks to the population increasing with increasing time interval to attack detection. (b) Timescale for deploying specific countermeasures in order for them to be effective against a CBW attack.

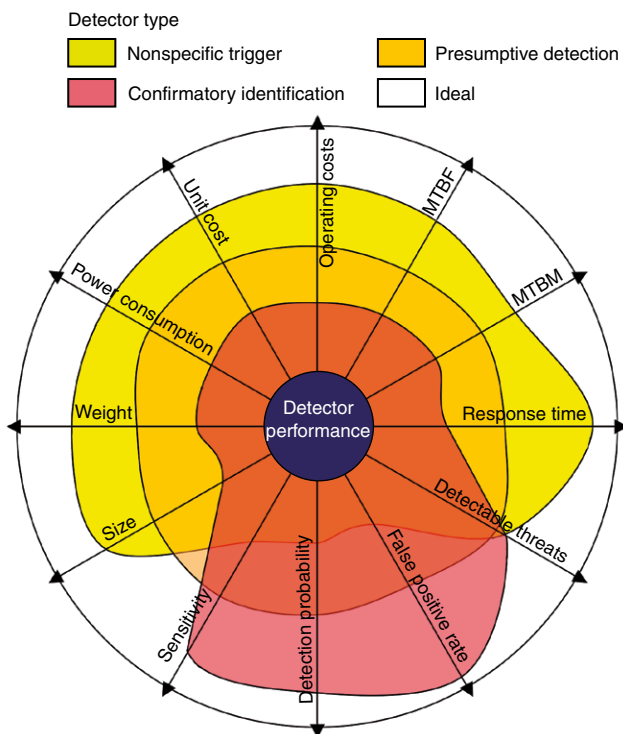


Figure 4. Notional spider chart plotting relative merits of detector types for 12 performance metrics (adapted from Ref. 27). The plot is nonlinear and not to scale, with ideal performance at the extremes of the plot and poor performance at the center of the plot. (MTBF and MTBM = mean time between failure and maintenance, respectively.)

CURRENT AND FUTURE CBW SENSORS

Remote CBW Sensors

There are two major categories of CBW sensors, depending on whether they are sensing remote (from several hundred meters to several kilometers) or local environments. The types of remote sensors may be active (e.g., lidar [light detection and ranging]),^{28–32} passive (most often multispectral, e.g., FTIR [Fourier transform infrared]),^{33–35} or differential optical absorption spectrometers.³⁶ Almost all remote (stand-off) sensor systems target the early detection and identification of chemical and/or biological aerosols (vapors and/or clouds), and as such they are prone to local weather/climate conditions at the time of measurement. For military defense applications where false alarm tolerance may be higher than in civilian applications, low-specificity remote sensing of bioaerosol clouds is the front line of defense. Here, as noted earlier, the significant science and technology challenge for these sensors is to elevate their specificity.

Several lidar systems for BW detection have been developed. Typically, pulsed lasers are used with wavelengths overlapping the characteristic dimensions of BW aerosols (from 1 to 10 μm), which allows efficient elastic backscattering of the laser light and provides

information on cloud density and spatial distribution. The methodology also involves measurement of the depolarization of the lidar return signals backscattered from a biological aerosol. In addition, inelastic scattering signals (e.g., UV-induced fluorescence or Raman) can be detected and used to provide information on the aerosol material through, for example, discrimination between bioaerosols and inorganic aerosols. Lidar sensors can detect the presence of an aerosol cloud at ranges up to 10 km and discriminate whether the cloud is biological or nonbiological at ranges of up to 4 km. The SINBAHD (Standoff Integrated Bioaerosol Active Hyperspectral Detection) program has investigated the sensitivity and discrimination capabilities of an inelastic lidar based on the intensified range-gated spectral detection of laser-induced fluorescence.³² A prototype lidar, based on a xenon fluoride excimer laser and image-intensified CCD detector, has shown a sensitivity of a few living bioaerosol particles per liter of air for a range of 1.4 km at night. Furthermore, good discrimination between two different microorganisms (*Bacillus subtilis* and *Erwinia herbicola*) has been demonstrated based on the spectral signatures of each microorganism.³²

Recently, a nonlinear lidar system that employs a mobile femtosecond laser, the Teramobile³⁷ (built by a French-German consortium), was tested and its ultrashort terawatt laser pulses were used to induce two-photon excited fluorescence in simulant particles at a remote location.³⁸ Extrapolation of these results to the detection of tryptophan (a strongly fluorescing amino acid present in microorganisms) suggested efficient detection of fewer than 1000 bioaerosol particles per liter at a distance of up to 2 km. A particular advantage of the terawatt femtosecond laser for remote sensing is the formation of an intense remote source of white light at an altitude of more than 20 km along the laser beam.³⁹ This source, an ionized region of air that emits a white-light supercontinuum covering the entire visible and near-IR ranges to around 4 μm , exhibits a directional behavior with enhanced backward scattering. The Teramobile thus may allow probing the chemical composition of clouds between the remote source and the observer.

Although passive remote sensing devices do not possess range resolution like lidar (achieved by the use of nanosecond to femtosecond pulses), they are typically much smaller and easier to install and operate in the field. Signals in a much broader spectral range can be acquired, but they are range integrated and require sophisticated algorithms for correction and background subtraction.⁴⁰ Carrieri has recently described the design and functional capabilities of the PANSPEC, a panoramic imaging IR spectroradiometer used as a chemical vapor sensor.³³ The system utilizes a camera and fused solid-state interferometer to collect and image ambient IR radiance from a panoramic field of view. In

addition, an active photopolarimeter provides a laser beam beacon that allows identification of feature spectra recorded by the interferometer. The capability of a passive FTIR sensor for remote detection of biological aerosols has also been investigated recently.^{34,35} Bioaerosols containing *B. subtilis* have been detected for the first time at a distance of 3 km in an open-air environment with very low thermal contrast between the aerosol and background brightness temperatures (≈ 1 K). This was achieved through the use of new hyperspectral detection, identification, and estimation algorithms based on radiative transfer theory.

Another evolving category of sensors, between remote and local, involves the use of an unmanned aerial vehicle or balloon to transport point detectors into an aerosol cloud. For instance, a small unmanned, all-electric aircraft, custom-built for the purpose of air-particle collection, was catapult-launched, flown by line of sight for a 20-min mission, and recovered after landing.⁴¹ The payload included a particle collector, a fluidics control unit, and a biosensor. During the trial, an aerosolized bacterial sample was successfully collected and remotely identified. An issue with the concept of operations of such an approach is the requirement for sufficiently early warning before vehicle launch.

Biological Agent Point Sensors

Sample Preparation

BW agents can be released in/via an array of mediums: aerosol/dust, water, soil, food, humans, etc. As discussed previously, biosensors must be capable of detecting and identifying the agent in trace concentrations in a wide variety of these mediums. Therefore, sample preparation has long been recognized as critical for the successful performance of any BW sensor platform. It has also been recognized that a single universal approach for sample preparation that can fit all possible BW deployment

scenarios is not yet realistic. Currently, sample preparation protocols for BW detection involve a set of sequential and/or parallel bioanalytical procedures (Fig. 5). A fully integrated sample preparation platform incorporating recent advances in microfluidics has been integrated into a system for autonomous detection of aerosolized *B. anthracis* and *Y. pestis*, two of the most lethal BW agents.⁴² In addition, Hindson et al.⁴³ have interfaced an automated sample preparation module with aerosol sampling and immunoassay-flow cytometric detection. This system demonstrated excellent stability for more than 5 days of unattended continuous operation.

A number of so-called micro total analysis systems (μ TAS) for bioanalytical/biosensor applications have been reviewed recently.^{44–46} These “labs on a chip” are typically monolithic devices etched in glass, silica, or molded plastic, and their operation is based on continuous flow in microchannels aided by diffusion, pressure gradient, electrophoresis, electroosmosis, etc. Broyles and coworkers⁴⁷ have demonstrated a number of microfabricated devices integrating sample filtration, solid-phase extraction, and chromatographic separation. Such devices have attractive features: miniaturization, a high degree of integration, high performance, fast response, and versatility.⁴⁸ It is argued that large and sophisticated instruments currently used for BW detection in the laboratory can be shrunk into field-deployable μ TAS sensors. For instance, microfluidics-based flow cytometry of intact bacteria (e.g., *E. coli*) was recently demonstrated by McClain and coworkers.⁴⁹ Zhou et al.⁵⁰ have recently developed a microfluidic chip system for severe acute respiratory syndrome (SARS) virus detection, which includes both polymerase chain reaction (PCR) for DNA amplification and capillary electrophoresis for sample preparation. Finally, Herr et al.⁵¹ have integrated polyacrylamide gels onto a μ TAS platform for electrophoresis-based immunoassays to detect bacterial toxin (tetanus)

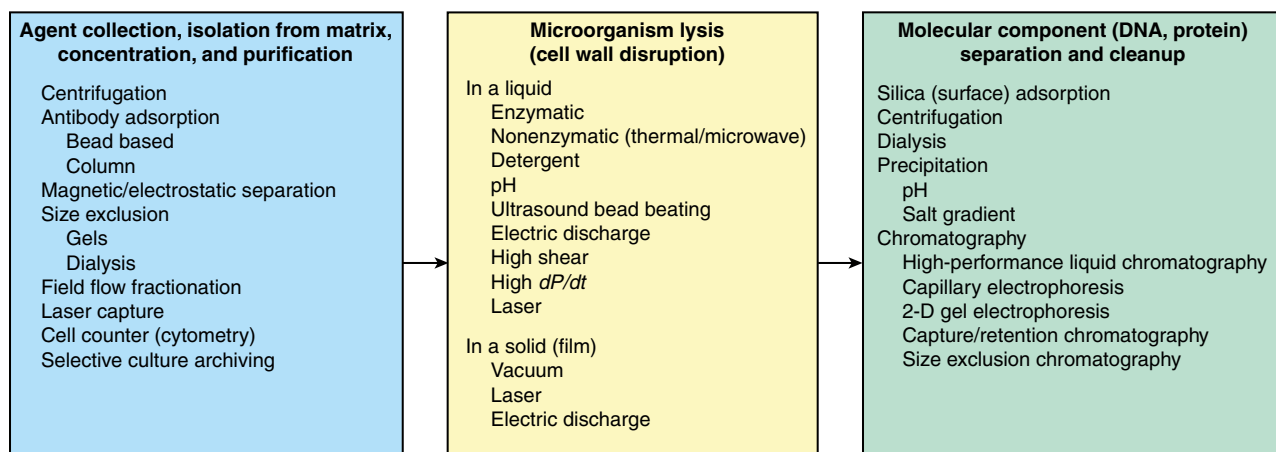


Figure 5. A set of typical bioanalytical procedures, grouped in three separate modules, that comprise the essential steps in CBW sample preparation protocols.

antibodies. Their assay, performed in buffer or diluted serum, can be completed in less than 3 min.

Nucleic Acid–based Sensors

Among the various molecular detection methodologies for BW, the most promising are the nucleic acid–based sensor approaches. These approaches stem from the capability to selectively amplify DNA molecular fragments using PCR. In addition, the spectacular advances in high-throughput, low-cost genome sequencing technologies⁵² have produced sequenced genomes for all major threat agents, enabling specific DNA-based detection using bioinformatics. The combination of PCR and bioinformatics has also led to the development of new therapeutics and vaccines.⁵³ Several reviews discussing the advantages and limitations of PCR—the most widely used technology for detection and identification of BW agents and molecular-based diagnostics of infectious diseases—have appeared recently.^{54,55} PCR-based techniques can be applied for both specific and broadband BW detection and are certainly more cost-effective and much faster than traditional microbiological approaches. Real-time PCR (RT-PCR) procedures based on monitoring the intensity of laser-induced fluorescence during the PCR target DNA amplification cycles are becoming sufficiently rapid and sensitive. For instance, RT-PCR can detect fewer than 53 *Bacillus* spores in a number of complex environmental, clinical, and food samples.⁵⁶ State-of-the-art RT-PCR has a sensitivity of four copies of smallpox virus target DNA per sample.⁵⁷ In addition, the RT-PCR method had a very high specificity: only smallpox virus DNA was detected, whereas similar viruses (several human herpesviruses as well as poxviruses other than orthopoxviruses) were not.⁵⁸

Oligonucleotide Microarrays

Oligonucleotide microarrays may offer a fast, high-throughput alternative for the parallel detection of BW and other microbial pathogens.^{59,60} Microarrays are sets of parallel, discrete, and spatially addressable probes on a solid substrate (DNA- or RNA-hybridization chips), where each probe is complementary to a target (pathogen-specific gene sequence). Typically, PCR amplifies each target (if present) and the products are then hybridized to the complementary probes on the array. For example, four orthopoxvirus species pathogenic for humans (variola, monkeypox, cowpox, and vaccinia viruses) were specifically detected and distinguished from chickenpox virus by such an approach.⁶¹ While it can take 3 hours per sample/array, it has been suggested that multiple samples can be tested in parallel on the same array. Vora et al.⁶² have noted that the need for front-end target-specific nucleic acid amplification constrains the advantages of the microarray-based approach. They recently evaluated the utility of

four different “broader-band” front-end amplification strategies for pathogenic *E. coli* O157:H7.⁶² All five diagnostic targets were detected in a spiked environmental water sample that contained a 63-fold excess of contaminating DNA. The performance of a universal nucleic acid sequence biosensor has also been described.⁶³

In the last few years, Mirzabekov and others have developed a series of 3-D gel-based microchips.^{64,65} Such microchips are currently fabricated by copolymerization of gel components and immobilized molecules. The immobilized capturing probes (e.g., DNA, proteins) are evenly distributed throughout the microchip gel element with a high yield, providing a 3-D reaction volume as opposed to other approaches where probes are bound to the chip surface (a 2-D reaction layer). Even bacteria and yeast cells can be immobilized in the gel while maintaining their viability. These oligonucleotide gel-based microchips are inexpensive and can be manufactured in large quantities. Such chips have been combined in a three-component system for microorganism identification.⁶⁵ The system comprises a minicolumn for successive DNA and RNA isolation, fractionation, fragmentation, and fluorescent labeling; microarrays of immobilized oligonucleotide probes for RNA or DNA identification; and an imager for detecting hybridization of fluorescently labeled fragments. The procedure is rapid: beginning with whole cells, it takes approximately 50 min. Chips have been developed for reliable identification of *Mycobacterium tuberculosis* and its antibiotic-resistant strains; orthopoxviruses, including the smallpox virus; and *B. anthracis*.

Mass Spectrometry

For more than two decades mass spectrometry (MS) has been an important tool for the detection and identification of CW in field settings as well as for verification and monitoring in compliance with the international convention for the nonproliferation and control of CW.^{66–68} Rapid gas chromatography (GC) methods,⁶⁹ either alone or in various combinations involving MS, FTIR,⁷⁰ or ion mobility spectrometry (IMS),^{71,72} have been developed for detection of volatile CW agents on the battlefield or in urban environments. Multiple analytical techniques to efficiently characterize a CW simulant have been described, including GC-MS, GC-FTIR-MS, as well as GC atomic emission detection.⁷³ In addition, newer atmospheric pressure ionization methods have been implemented with a variety of mass spectrometers for analysis of low volatile organic compounds (drugs, explosives, CW simulants, intact bacterial cells, etc.) under atmospheric pressure conditions desorbed from a variety of surfaces. These methods—DESI (desorption electrospray ionization)⁷⁴ and DART (direct analysis in real time)⁷⁵—promise to significantly improve the types of MS-based sensors for rapid and sensitive CBW detection.

Recently, various types of mass spectrometers have received considerable attention as a method for the rapid and highly reliable detection of microorganisms.⁷⁶ In particular, MALDI (matrix-assisted laser desorption/ionization) MS has been demonstrated as an efficient and sensitive tool to detect and identify intact microorganisms such as viruses, vegetative bacteria and bacterial spores, and fungi.^{77,78} MS as a method for microorganism identification has several advantages. It is rapid (a typical experiment, including sample collection and sample preparation, takes minutes versus days for classical microbiology experiments). It is broadband, i.e., it can detect not only microorganisms, but protein and nonprotein toxins (e.g., lower-mass nonvolatile substances such as saxitoxin and palitoxin). This last feature distinguishes MS from all DNA-based technologies, which require the presence of DNA from the producing organism (e.g., castor plant) to infer the presence of a particular toxin (e.g., ricin). The combination of various types of MS into a single ("universal") sensor for both volatile and nonvolatile CW and BW has been proposed.¹⁵ MS is sensitive as well; typically, a signal with a sufficient signal-to-noise ratio can be generated from a sample containing fewer than 10^4 organisms or a few femtomoles of a toxin. It can also be interfaced to a variety of sample collection and sample processing modules to allow versatile sampling from different environments (aerosols, liquids, powders). MS is easily automated and computer friendly; the latest developments in bioinformatics and genome databases can be coupled to MS experimental data for the robust identification of microorganisms.⁷⁹ Furthermore, MS instruments can be miniaturized.⁷⁹ MALDI time-of-flight (TOF) instruments for BW detection have been described that fit, e.g., into a regular suitcase for field-portable use.^{80,81} Depending on the particular deployment scenario, these MALDI-TOF instruments are equipped with sample collection/processing devices for either aerosol or solid samples.

A combined laser fluorescence/laser ionization TOF mass spectrometer has been evaluated recently for real-time detection and identification of individual aerosolized microbial particles, e.g., spores of two *Bacillus* species (*B. thuringiensis* and *B. atrophaeus*)⁸² or *M. tuberculosis* bacteria.⁸³ The approach is reagent-less, i.e., no sample preparation is required. Only lower-mass ($<m/z$ 200) positive and negative ions are ablated and detected. The two *Bacillus* spore species are distinguished from one another and from the other biological and nonbiological background materials, with no false positives, at a sensitivity of 92%.

An entirely different approach for BW detection, combining nucleic acid detection with MS, has been described recently.⁸⁴ In this approach, analysis of PCR-amplified variable regions of microbial genomes is performed by electrospray ionization MS. The approach is termed TIGER (triangulation identification for the

genetic evaluation of risks) and relies on "intelligent PCR primers" to target broadly conserved regions that flank the variable genome regions. The sample preparation procedure takes more than 1 hour. The masses of PCR products with lengths between 80 and 140 base pairs must be determined with an accuracy of better than 20 parts per million (i.e., better than ± 0.35 Da for a 35-kDa molecule). This allows unambiguous assignment of the base composition of the amplified regions, which should unequivocally determine the microorganism by comparison with its genome sequence. Although appealing, so far the TIGER approach has been demonstrated only on an FT ion cyclotron resonance mass spectrometer with a superconducting magnet, a device that can be used only under specialized laboratory conditions.

SITUATION AWARENESS FOR PROTECTING HUMAN POPULATIONS

Multisensor information fusion is critical to detecting and responding to human disease outbreaks (natural or otherwise). This approach compares sensor data, human health indicators, and other available information.

There are three stages in which an infection can be detected in a person: incubation stage (asymptomatic), prodromal stage (early symptomatic), and advanced stages. The ability to identify individuals in the incubation stage would be useful when a biological attack has been detected or suspected and those potentially infected can be screened for treatment and possibly quarantine. Recent research is investigating the possibility of analyzing exhaled human breath for indications of upper respiratory infection.⁸⁵

With no direct detection or suspicion of a released BW agent, the biological attack must be detected in the infected and symptomatic population during the prodromal or advanced stages of infection. The public health community has traditionally detected disease outbreaks through disease surveillance by mandating that health care providers report the diagnosis of diseases that pose an unusual public health risk.⁸⁶ This approach generally detects diseases in the advanced stages, when the symptoms of the disease, or laboratory test results, allow a definitive diagnosis. It is inadequate for many potential bioterror agents because of the reduced efficacy of treatments after the early stages of the disease. For example, anthrax is more successfully treated when antibiotics are administered 2 days before the disease is typically diagnosed.

If a bioterror disease outbreak can be detected in the prodromal stage using prediagnostic health care data, the early response of the public health community can save many lives. Toward this end, the civilian and military public health communities have been developing systems to collect, examine, and interpret aggregated consumer and prediagnostic medical transactions to look for

an anomalously high consumption of health care that is consistent with a covert biological attack. The data are typically cleansed of identifying personal information to protect privacy. This approach has been termed “syndromic surveillance,” since the data reflect disease symptoms rather than diagnosis. Several systems have been developed and are deployed across the country^{87–89} and at U.S. military installations around the world.⁸⁷

The challenge of syndromic surveillance has been to develop alerting algorithms to detect changes in the data caused by a bioterror attack while ignoring the natural fluctuations of the background data. Attack-induced changes in the data will depend on the attack scenario (e.g., agent, method of dissemination, amount, location, date and time, duration) and the attack environment (e.g., local weather conditions, commuting and travel patterns, levels of endemic disease, spatial distribution of the population). The natural fluctuations of the background data are the result of systematic variability caused by endemic diseases, day-of-week and holiday effects, promotional discounts on over-the-counter pharmaceuticals, seasonal variations, weather, and the environment, along with the statistical variability from counting health care transactions. In analogy to physical detectors, the detection sensitivity of the alerting algorithms, i.e., the ability to detect changes in the data resulting from bioterror attacks, must be traded against the detection specificity, i.e., the ability to ignore background fluctuations. The use of environmental sensor data (ozone levels, pollution, pollen counts) has the potential to further improve the specificity of biosurveillance-based detection systems. For example, upsurges in the purchase of respiratory medications, which could indicate a possible early anthrax outbreak, can sometimes be “explained away” by detecting environmental factors (high ozone levels) known to trigger respiratory symptoms in asthmatics and other sensitive groups.

In syndromic surveillance, the individual health care transactions are aggregated into syndrome groups designed to emphasize the expected changes in the data caused by the disease outbreak relative to the background fluctuations. Anomalies are detected both temporally, in the day-to-day changes in the counts, and spatially, in abnormal geographic distributions of health care transactions. With all of the various aggregations of the data being scanned for anomalies, the next challenge of syndromic surveillance is to reduce the amount of information being presented to public health personnel. The problem will only be exacerbated as data streams are being collected from additional sources. Each additional aggregation or alerting algorithm contributes to the false alert rate of the system, eventually causing an unmanageable workload for public health personnel investigating each alert. Multivariate detectors⁹⁰ and data fusion approaches^{91,92} are being applied to meet this challenge.

The key is to refine both the computational models for expected changes in the data due to an attack (e.g., by targeting high-probability scenarios) and systematic fluctuations in the data (e.g., by including the variables known to affect the background data).

RECENT CHEMICAL AND BIOLOGICAL WEAPONS DEFENSE-RELATED RESEARCH AT APL

APL scientists and engineers have been developing sensors and systems to detect CBW attacks since well before 9/11. A selected subset of these efforts has been reviewed earlier in special issues of this journal.^{93–95} Several specific research and development efforts are currently under way to address a number of the previously highlighted technology challenges. Among various sensor platforms, APL is developing sensors for direct CW and BW detection using molecularly imprinted polymers⁹⁶ and TOF MS,^{15,78,80,81,97,98} respectively. The Laboratory has also been a leader in developing the testing and evaluation methodologies for CBW detection systems.⁹⁹ Approaches to mid-term BW response by monitoring human infections via breath analysis have been described,⁸⁵ and BW detection/protection through surveillance of prediagnostic medical data of large populations has been discussed.^{100–102} Efforts at APL include evaluation and characterization of a variety of other CBW sensor systems, e.g., RNA/DNA gel-based chips for BW pathogens¹⁰³ as well as their extension for identification of virulence factors and detection of non-sequenced pathogens. In addition, theoretical bioinformatics modeling to extend the DNA chip approaches for detection of emergent and bioengineered biological threats has been initiated. Other significant efforts at APL include improving the specificity of standoff bio-aerosol detection systems,^{31,104,105} sensor systems and methods for detection of low-volatility chemical compounds, methods for BW decontamination¹⁰⁶ and indoor space protection,¹⁰⁷ and information fusion approaches for incorporating sensor data and other information into syndromic surveillance systems.⁹¹

Finally, APL is a major partner in the new Center for Preparedness and Catastrophic Event Response (PACER) hosted at JHU for the U.S. Department of Homeland Security. The aims of the center are to advance the state of the art in CBW defense and to all response hazards and to develop the academic discipline of PACER to educate future generations. Addressing the challenges in the rigorous characterization of biosensor performance will be one of the APL-led initiatives in this new center.

ACKNOWLEDGMENTS: This article reflects the numerous and productive interactions among the authors and scientists in multiple APL departments

(most significantly the National Security Technology Department and the Research and Technology Development Center). We gratefully acknowledge all our colleagues (too numerous to list here) who have been diligently working in this area long before our own involvement in these various efforts. We thank specifically R. Benson, H. Burkom, B. Collins, H. Heaton, J. Jackman, H. Ko, J. Lombardo, J. Miragliotta, R. Potember, C. Schumacher, and P. Smith for helpful suggestions on the manuscript.

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