Technologies for the Rapid and Non-invasive Detection of Infectious Disease at U.S. Ports of Entry

Alena James & Luther Lindler

The 2014 Ebola outbreak in West Africa captured the nation's attention, highlighting the need for improved biosurveillance at the border and identifying a capability gap in the United States' homeland defense and security architecture. In response, the U.S. strove to fill knowledge gaps and operational capabilities by advancing experimental patient treatments and therapies, strengthening the traveler screening process, and enabling a national strategic response framework for infectious disease—working with the United Nations and states of Liberia, Sierra Leone, and Guinea to contain the epidemic [1]. Through the execution of Operation United Assistance, the U.S. Department of Defense (DoD) dedicated more than $2.3 billon to the deployment of medical military personnel, research labs, hospital beds, and other necessary medical supplies to help more than 28,000 West Africans affected by the outbreak [2]. In addition, the Centers for Disease Control and Prevention (CDC), Department of Homeland Security (DHS), and West African governments implemented traveler health screenings at ports of entry (POE) to fortify border security and contain the spread of the virus.

To enact a risk-based screening process at domestic POEs (see Figure 1), officers from U.S. Customs and Border Protection (CBP) assessed travelers entering the country for potential exposure to Ebola. High-risk travelers—including those entering from the three affected African states [3]—received an enhanced health screening, in which a non-contact infrared thermometer was used to measure their temperature. Travelers with temperatures greater than 100 degrees Fahrenheit were assessed further and then advised to seek medical care [4–10].

In October 2014, after CDC confirmed the first case of Ebola in the United States, CBP began to implement an enhanced entry screening program. In the first 30 days of the program, CBP officers screened 1,993 travelers from Guinea, Liberia, and Sierra Leone. CBP referred 86 of those travelers to the CDC for a more intensive health screening [3]. Of those, seven asymptomatic individuals were sent for further medical evaluation [3]. While deployed, U.S. military personnel serving in Operation United Assistance were also monitored daily for exposure symptoms [11, 12].

Before re-entering the U.S., non-symptomatic DoD personnel received additional medical screening and were placed in controlled monitoring for 21 days [11, 12]. During this time, military personnel with no known exposure were assessed for febrile symptoms, measuring their temperature twice daily [12]. DoD civilians supporting the deployments (but without known exposure) were permitted to either voluntarily participate in DoD's controlled monitoring protocol, or follow guidance from CDC, state, and local public health authorities on the active monitoring of Ebola [12].

During the outbreak, border screenings of travelers were essential to preventing the biothreat from spreading to other countries [13]. More than 38,000 travelers entering the U.S. underwent enhanced Ebola screening [14]. Apart from the results of traveler questionnaires taken during CBP's secondary screening stage, the only other determining risk factors were symptoms of infection—namely an elevated temperature. Of the 38,000 travelers screened in this manner, 2,975 returned positive responses to the questionnaire, 81 travelers presented elevated temperatures, 2,996 travelers were referred to tertiary screening, and 49 travelers were sent to medical facilities for further assessment [4].

Viral hemorrhagic diseases like Ebola can be spread via direct contact with bodily fluids—including saliva, sweat, blood, vomit, and waste excrement [15]. Presentation of high fever is actively used to confirm outbreaks at POEs, as fever can be an indicator that a traveler's body is experiencing an infection. Even so, fever cannot be used as a diagnostic marker for specific pathogens or diseases, because it is a common clinical symptom.

However, the only diagnostic tool currently available for secondary screening in CBP's tiered process is a thermometer. The Ebola outbreak reinforced the need to deliver a field-deployable diagnostic test capable of rapidly and non-invasively screening pre-symptomatic travelers for diseases of concern.

Development of a diagnostic tool for this stage could expedite the screening process, improve response times for positive tests, and provide public health officials with better information than current questionnaire- and temperature-based tools. Moreover, with a severe biothreat like Ebola, the use of non-invasive diagnostics is critical for protecting medical screening officers (and other travelers) from exposure. Non-invasive sampling collection techniques are defined as such because they do not penetrate through the skin. Examples include nasal, oral, and epidermal swabs, or the collection of breath samples.

Required characteristics for an effective POE screening device include: a high degree of portability, user friendliness, durability, non-invasive sample collection, generation of minimal hazardous material during the analysis process, accurate diagnostics for multiple diseases in less than 60 seconds (a standard benchmark for "rapid" analysis), and the ability to detect a pathogen in an asymptomatic traveler.

This article reviews four distinct technologies that can be applied to improving research and development (R&D) in the field of rapid diagnostics. These four technologies include nucleic acid-based detection technology; antigen-antibody binding-based detection technology; volatile organic compound-based detection technology; and infrared light-based detection technology. A review of the literature suggests that current R&D for improving the performance of rapid diagnostic tests is focused heavily on tests rooted in nucleic acid-based and antigen-antibody binding-based detection technologies (see Figure 2) [16–19]. Less emphasis and attention have been paid to the development of volatile organic compound-based and infrared light-based detection technologies.

Testing for Diseases of Public Health Significance

Four classes of disease can be closely monitored and detected using traditional screening practices (e.g., measuring temperature, heart rate, or other symptoms). These four classes include infectious respiratory, viral hemorrhagic, vector-borne,
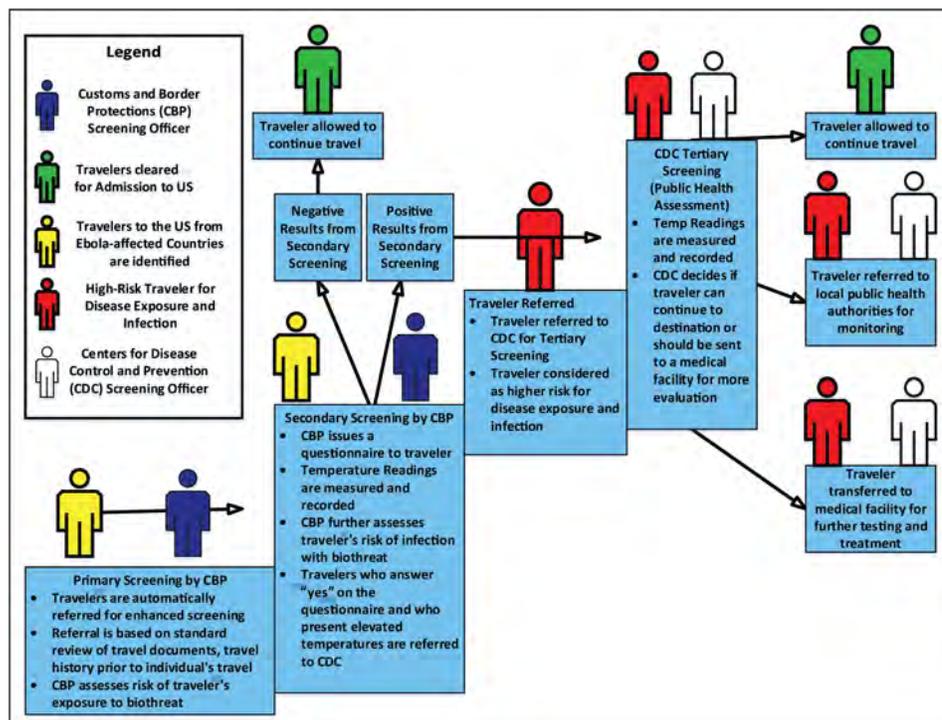

Figure 1. Risk-based screening process for travelers entering the U.S. During the 2014 Ebola Outbreak. This figure demonstrates the traveler screening process used to assess travelers to the United States during the 2014 Ebola outbreak [4–10].

and gastrointestinal disease. Foreign nationals seeking to enter the U.S. under immigration or refugee protocols must pass a medical examination as part of the visa application process, and those who present symptoms of what CDC terms "communicable diseases of public health significance" are excludable from entry [20]. Foreign nationals visiting or transiting through the U.S. who present symptoms of such diseases may also be examined before entry is granted. U.S. citizens re-entering the country are not required to pass a health screening to gain entry, except in the event of an emerging disease outbreak posing a significant threat to the nation's public health.

Diagnostic tests vary in their approach to disease detection, but many are designed to target an analyte specific to a pathogen that can be bound to some form of a bioreceptor. After binding, a transducer can be used to amplify a signal that indicates the binding of the analyte to the receptor. Diagnostic tests designed in this manner are crucial for pathogen detection *in vitro* and allow medical professionals to select an appropriate treatment regimen. Diagnostic technologies also vary in their processing times, instrumentation reliability, and trade-offs between specificity and sensitivity.

Nucleic Acid-based Detection

Nucleic acid-based detection technologies use genetic code to identify pathogens. While doing so, nucleic acid-based detectors will target chemical compounds within either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Several technologies, such as reverse transcriptase-polymerase chain reaction (RT-PCR), PCR, and real-time PCR (qPCR), can detect and confirm multiple pathogens through nucleic acid amplification and quantification. Many Ebola diagnostic assays used during the 2014 outbreak were developed on the principles of RT-PCR [21]. While these lab-based methods are the most accurate available, they require expensive instrumentation, a high degree of technical expertise, invasive sampling techniques (e.g., venipuncture), and lengthy processing times (two or more hours) to operate. However, nucleic acid amplification provides a thorough analysis of the amplified products. Targeted amplification, combined with nucleic acid sequencing, can provide more detail about a biological agent, po-
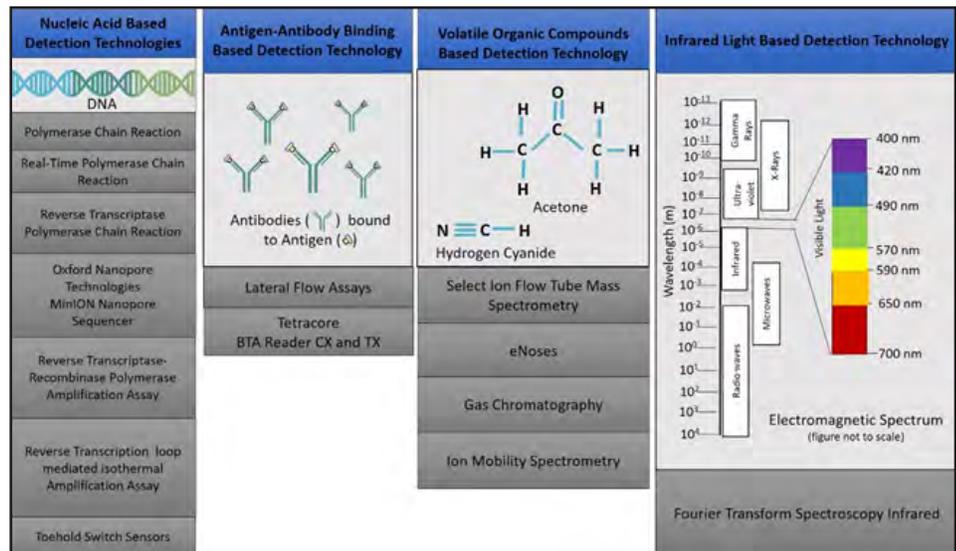

Figure 2. Technological approaches for pathogen detection. This figure depicts existing technologies with the potential to further advance the development of rapid and non-invasive diagnostic tests [16–19].

tentially including its relationship to another biothreat, or its susceptibility to medical countermeasures.

Through technical R&D, the diagnostics industry has improved our ability to complete sequencing in a field-deployable (non-laboratory) environment. For example, Oxford Nanopore Technologies Ltd. developed the MinION Nanopore Sequencer, which shows promise for use at POE. The MinION has demonstrated its ability to rapidly sequence viral genomes during the early phases of an influenza pandemic [22] and during the Zika virus epidemic [23]; and it was used by researchers in Guinea during the Ebola outbreak to process serum samples from patients in real time [24]. Powered by a USB port, the MinION relies on a flow cell to analyze nucleotide bases and it can simultaneously run multiple samples. The sequencer can operate independent of an internet connection and process a sample within an hour. Using the MinION, researchers have conducted real-time bioinformatics analysis in resource-limited areas such as the rain forest [25]. It has also been used in the rapid metagenomic detection of Chikungunya virus, Ebola, and hepatitis C virus in human blood samples [26]. These features make the MinION a promising candidate for border medical screening, if the requirement for non-invasive sample collection is waived.

Reverse transcriptase-recombinase polymerase amplification (RT-RPA) is another nucleic acid-based approach for pathogen detection. These assays function by plac-

ing enzymes and the DNA or RNA sample of interest within a centrifuge to initiate the amplification reaction (which can take place at room temperature). RT-RPAs have been useful in the on-site detection of dengue virus in human serum samples [27]; in the laboratory-based detection of multiple strains of Rift Valley fever virus taken from virus cultures [28]; and in the on-site detection of yellow fever virus taken from cultures, mosquito pools, and human plasma samples [29]. These assays benefit from the fact that the reaction does not require a thermocycler, which minimizes assay times and expense. These assays have also been used to identify viruses in pigs such as type 2 porcine reproductive and respirator syndrome virus [30], and detect epidemic human norovirus strains in viral RNA [31]. Reverse transcription loop-mediated isothermal amplification assays employ a similar technique, and have been proven to identify Middle East Respiratory Syndrome Coronavirus in human samples [32].

Synthetic biology—the generation of new biological products like genes, cells, or enzymes using advanced bioengineering techniques—has also furthered the development of nucleic acid-based detection technologies. For example, the development of programmable RNA sensors has led to the production of paper-based methods for virus detection. Such methods combine RNA sensors, known as Toehold Switches, with a freeze-dried, cell-free protein expression paper platform [33]. These methods are capable of identifying Zika virus without reporting a false detection of

the closely related dengue virus [33]. As a review of the literature indicates, R&D on nucleic acid-based detection technology continues to be the primary focus in the advancement of rapid diagnostic tests.

Antigen-antibody Binding-based Detection

Relied on as diagnostic instruments since the early 1990s, antigen-antibody binding-based detection technologies work rapidly and are easy to interpret. The most relied-upon assays are Lateral Flow Assays (LFAs), which are relatively inexpensive and user-friendly [34]. Antigen-antibody binding diagnostic tests use blood proteins (antibodies) to capture foreign substances from pathogens (antigens). Antigen-antibody binding is specific; this means that only certain antibodies can bind to certain antigens. For a disease-detecting assay to function, strips are coated with pathogen-specific antibodies. Once a sample is applied, antigens flow toward the antibodies, which are coated in gold or latex particles. They bind and form complexes that accumulate in a line, yielding a colorimetric result that indicates the absence or presence of the pathogen. LFAs' user-friendliness and cost-efficiency make them appealing tools for medical screening in non-laboratory environments.

However, LFAs can be limited in their analytical capabilities due to sensitivity and specificity challenges. Many LFA test strips are singleplex, or single-pathogen-specific. This is problematic when attempting to detect the presence of unidentified (or multiple) pathogens. Recent efforts have produced multiplex LFAs capable of detecting multiple pathogens simultaneously. Researchers have demonstrated the multiplex detection of 10 epidemic foodborne pathogens, including *Escherichia coli*, *Salmonella*, and *Cholera* [34]. However, multiplex LFAs face challenges like high levels of cross-reactivity (non-specific binding to antibodies), which can produce false positives among multiple pathogens [35]. Often used in environmental sampling, LFAs have identified the presence of biothreat agents like *Bacillus anthracis* [36]. Furthermore, these types of tests have been integrated with the use of mobile devices (e.g., smartphones, tablets) in analyzing results. For example, Biothreat Alert Multiplex Strips, produced by Tetracore, target up to five different antigen targets at once:

a control line, *Yersinia pestis*, *Francisella tularensis*, *Bacillus anthracis*, and *Burkholderia*. These strips can be read and analyzed by Tetracore's BTA Reader CX and BTA Reader TX phone or tablet add-ons to confirm the identity of the pathogens within 60 seconds [37].

Given their limitations regarding sample collection and multiplex-use, LFAs are only moderately suitable for the biomedical screening of travelers at POEs. In comparison to nucleic acid-based diagnostic assays, LFAs are more cost-effective and require less scientific expertise for use. However, when used outside of a laboratory environment, antigen-antibody tests are susceptible to factors that can compromise their reliability. Variables like temperature, light, and pH can compromise an LFA's results. Assay performance is also affected by the shelf life and storage conditions of the testing reagents.

Volatile Organic Compound-based Detection

Defined in a medical context, volatile organic compounds (VOCs) are small molecules produced by the body (and/or microbial pathogens) and liberated in bodily fluids like breath, urine, feces, and sweat [38]. Research on the viability of using VOCs as indicators for infections is underway, but the scientific literature has already recognized an association of several VOC arrays with specific diseases. Urinary tract, gastro-intestinal, fungal, and bacterial infections, such as *Mycobacterium tuberculosis*, are just a few examples of instances where VOCs can be used as markers for the detection of disease [39]. Studies have also linked certain concentrations of acetone in breath to diabetes mellitus [40]; increased levels of hydrogen cyanide in the breath of patients aged seven to 17 to lung infections induced by *Pseudomonas aerugnosa* [41]; and the presence of thioethers in breath to *Plasmodium falciparum*, the protozoan parasite that causes malaria in humans [42].

In addition, several VOC-releasing bacterial pathogens—such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterobacter faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*—are being studied in order to produce statistical models of VOC–pathogen production relationships [43].

VOC analysis relies on the use of traditional chemical analytical technologies such as gas chromatography coupled with mass spectrometry (GC-MS) for compound identification. GC-MS is the gold standard for gas sample analysis, and it can analyze breath-borne compounds. Studies have used GC-MS in the detection of chemicals like ammonia, which, when present in breath, correlates with *Helicobacter pylori* infection in humans [44]. Other types of spectrometry that can be used to analyze chemical compounds include ion mobility spectrometry (IMS), selected ion flow tube mass spectrometry (SIFT-MS), and proton transfer spectrometry (PTS). IMS and SIFT-MS can provide real-time measurements of VOCs, and PTS has been used to detect VOCs associated with food spoilage-related bacteria [45]. All of these technologies provide a strong platform for the chemical analysis of gas samples.

Electronic noses, or e-noses, are sensors used to continually detect the presence of VOCs in gas samples [46]. E-nose technology relies on electronic sensors to identify patterns and recognize odors, but also typically integrates traditional chemical analyzers (like gas chromatography) into the system. Several e-nose devices are being improved upon or optimized for detecting the presence of disease-associated VOCs in human breath profiles [47]. Airborne chemical detection has already been deployed in efforts to interdict illegal substances and contraband at national borders [48], and tools for VOC detection are showing promise for use in the interdiction of human trafficking activity [49]. Recognizing that canines' exceptional sense of smell grants them the ability to sniff out certain chemicals and biomarkers, researchers have undertaken studies to better understand the flow of air within a dog's nose, to inform the development of better e-nose devices [50, 51].

Analysis of VOCs in human breath shows significant potential for swiftly and accurately identifying travelers infected with a disease of concern during a medical screening. The release of VOCs in the gaseous phase makes these compounds a non-invasive means of sample collection [52, 53]. However, the release of relevant biomarker VOCs may not occur until a person is in the very late stages of infection, making detection challenging during the pre-symptomatic stage. As additional R&D

focuses on exploring VOCs as markers for infectious disease, development of a portable, non-invasive VOC diagnostic tool may be plausible in the near future.

Infrared Light-based Detection

Like VOC sampling, infrared light also holds promise for use as a diagnostic technique—and it may be the most ideal technology for the development of a rapid, non-invasive detection device. Infrared light is often used in research laboratories as an analytical tool for determining the chemical components of solid or liquid samples. In comparison to ultraviolet radiation or visual light, infrared light reflects lower energy levels when applied to a sample [54], thus decreasing the risk of damage.

At present, no diagnostic test for an infectious disease uses this technology *in vivo*. However, infrared light-based detection devices have been deployed as biosurveillance scanners and used to detect febrile symptoms when screening travelers during outbreaks. Infrared thermal imaging scanners were used, with varying levels of effectiveness, to identify travelers presenting fevers during several seasonal flu outbreaks [55]; to complement other detection measures during the 2003 Severe Acute Respiratory Syndrome (SARS) outbreak [56]; and during the 2014 Ebola outbreak. The use of infrared thermal imaging scanners and non-contact infrared thermometers allows border officials to assess whether a given traveler may pose a risk of pathogen infection. However, it is important to note that non-contact thermometers have displayed limited efficacy in detecting the early stages of viral infections (such as influenza) in travelers [57].

Currently, engineers are working to apply this non-invasive technology to the development of user-friendly medical diagnostic instruments. In an ideal rapid and non-invasive diagnostic device, the instrument should be capable of directing light waves through the epidermal layer of the skin to detect the presence of a pathogen—without damaging the body. However, this diagnostic capability is not yet feasible, and the body of scientific literature related to its maturation remains limited in size. Direct application of infrared light to the body faces significant technical challenges, such as interference by dense physical features

of the skin that inhibit optical analysis. Skin pigmentation and thickness all influence the propagation of light waves, which in turn impacts the accuracy of optical measurements [58].

While there is no light-based detection technology available for diagnosis *in vivo*, there is a laboratory technique used to analyze samples for bacterial and viral presence. This technique, known as Fourier transform infrared spectroscopy (FTIR), uses vibrational spectroscopy to examine vibrations between molecules. FTIR relies on infrared radiation to analyze the chemical composition of samples, and it is the vibrational spectroscopic technique most widely used in bacterial detection [59]. FTIR technology can identify structural information, like molecular changes to cellular components, allowing for the rapid identification of bacteria. To date, this technology has successfully detected *E. coli O157:H7* in apple juice [60]; has been applied as a diagnostic and surveillance tool for cancer [61]; and has been demonstrated as a rapid technique for the identification of bacteria like *P. aeruginosa* in sputum samples [62]. In addition, FTIR has also successfully sensed foodborne pathogens such as *Listeria monocytogenes* [63].

Studies are still needed regarding the use of FTIR in rapid diagnostics in the biomedical field. Some researchers have had success using near-infrared light waves to monitor glucose levels in individuals with diabetes (or susceptible to it) [64]. Scientists have also attempted to use near-infrared light spectroscopy to detect and study viral or bacterial particles in model systems. In May 2018, scientists reported the use of near-infrared spectroscopy to detect Zika virus in *Aedes aegypti* mosquitoes [65]. Also, influenza and Ebola have been detected in serum samples via infrared light technology [66].

Because they are subject to the same challenges observed with nucleic acid-based, antigen-antibody binding-based, and VOC-based detection technologies, infrared light-based detection is still costly and not well-suited for field environments. Even so, infrared methods require no sample preparation, and results can be produced quickly in the form of spectral images. More research on integrating spectroscopy techniques with disease diagnosis procedures may lead to hybridized diagnostic devices

capable of non-invasively testing for a disease-causing pathogen, and providing an accurate diagnosis for further action.

Conclusion

The greatest challenge in developing a rapid diagnostic tool is designing a non-invasive sampling technique that can reliably identify pathogen carriers before they show symptoms. Traditional oral or nasopharyngeal swab specimen collection remains the least invasive method currently available for sampling. However, this technique still subjects the sample to lengthy processing times. The use of swabs generates hazardous waste, further increasing the risk of exposure and the spread of disease. The operational environment at a POE requires safer techniques, less costly, more specific, and more portable technologies to improve the medical screening process of travelers deemed high-risk for exposure during a biological outbreak. The use of VOCs and infrared light-based detection devices hold the most promise for meeting these criteria.

Travelers entering the country and warfighters returning to the U.S. after serving in areas with ongoing infectious disease outbreaks require extensive health screenings to ensure their safety and to protect the country against an outbreak. Continued R&D in improved rapid and non-invasive diagnostic technologies may enhance our nation's ability to prevent dangerous biothreats from entering the U.S., while providing timely and effective treatment for military and government personnel at risk of exposure.

Disclaimer

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Alena James
Chemical and Biological Defense ORISE Fellow, Engility Corporation

Upon the completion of this article, Alena James was a Chemical and Biological Defense ORISE Fellow at The Department of Homeland Security Science & Technology Directorate (DHS S&T). Recently, Alena transitioned to a new role as a science and technology advisor for Engility supporting the Department of Defense. She earned her M.S. in Biodefense from George Mason University, her M.S. and B.S. in Biology from Winthrop University, and her B.A. in Political Science from Winthrop University. Her research interests are in chemical and biological defense research in risk analysis, disease surveillance, border security, and technology development.

Luther Lindler, Ph.D.
Senior Science Advisor Bio-Programs (ST), Department of Homeland Security Science & Technology Directorate

Luther Lindler, Ph.D., joined the Department of Homeland Security (DHS) Science and Technology Directorate (S&T) in October 2003 as a Senior Science Advisor. He currently serves as the Chief Scientist for biological programs providing expertise in the area of infectious disease threats from a global perspective. He is responsible for developing new technology strategies to meet DHS mission needs in the area of defense against bioterrorism. Before joining DHS, he was a leader in the U.S. Army Biodefense program. Lindler holds a Ph.D. in Microbiology from the Medical College of Virginia, a M.S. in Microbiology from Clemson University, and a B.S. in Medical Technology from Lenoir Rhyne College in North Carolina. He is the principal editor of the book, *Biological Weapons Defense: Infectious Diseases and Counterbioterrorism.*