PERSPECTIVES

INTERNATIONAL MICROBIOLOGY (2014) 17:119-129
doi:10.2436/20.1501.01.214 ISSN (print): 1139-6709. e-ISSN: 1618-1095
www.im.microbios.org

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

CDC Report on the Potential Exposure to Anthrax*

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Executive Summary

The Centers for Disease Control and Prevention (CDC) conducted an internal review of an incident that involved an unintentional release of potentially viable anthrax within its Roybal Campus, in Atlanta, Georgia. On June 5, 2014, a laboratory scientist in the Bioterrorism Rapid Response and Advanced Technology (BRRAT) laboratory prepared extracts from a panel of eight bacterial select agents, including *Bacillus anthracis* (*B. anthracis*), under biosafety level (BSL) 3 containment conditions. These samples were being prepared for analysis using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, a technology that can be used for rapid bacterial species identification.

What Happened

This protein extraction procedure was being evaluated as part of a preliminary assessment of whether MALDI-TOF mass spectrometry could provide a faster way to detect anthrax compared to conventional methods and could be utilized by emergency response laboratories. After chemical treatment for 10 minutes and extraction, the samples were checked for sterility by plating portions of them on bacterial growth media. When no growth was observed on sterility plates after 24 hours, the remaining samples, which had been held in the chemical solution for 24 hours, were moved to CDC BSL-2 laboratories. On June 13, 2014, a laboratory scientist in the BRRAT laboratory BSL-3 lab observed unexpected growth on the anthrax sterility plate. While the specimens plated on this plate had only been treated for 10 minutes as opposed to the 24 hours of treatment of specimens sent outside of the BSL-3 lab, this nonetheless indicated that the *B. anthracis* sample extract may not have been sterile when transferred to BSL-2 laboratories.

Why the Incident Happened

The overriding factor contributing to this incident was the lack of an approved, written study plan reviewed by senior staff or scientific leadership to ensure that the research design was appropriate and met all laboratory safety requirements.

Several additional factors contributed to the incident:

- Use of unapproved sterilization techniques
- Transfer of material not confirmed to be inactive

*NOTE: On July 21, 2014, Timothy J. Donohue, President of the American Society for Microbiology, and Ronald M. Atlas, Chair of the Public and Scientific Affairs Board of the ASM, sent to all ASM members a letter [http://www.asm.org/index.php/public-policy/93-policy/93014-biosafety-7-14] concerning the recent events at the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH), which are documented in the CDC Report issued on July 11, 2014, that INTERNATIONAL MICROBIOLOGY reproduces here (the original can be accessed through the following link [http://www.cdc.gov/od/science/integrity/docs/Final_Anthrax_Report.pdf]). On July 31, Donahue and Atlas sent a second letter [http://www.asm.org/index.php/clinical-microbiology-update/137-policy/documents/statements-and-testimony/93024-durc-7-31-14] stating that "The ASM has a long history of supporting and informing public policy that is based on the essential principle of ensuring protection of public health and safety without unduly encumbering legitimate fundamental scientific research, clinical and diagnostic testing for the treatment and prevention of infectious diseases. The ASM has and continues to have a position that microbiological research must be done safely and in accordance with regulations governing the proper conduct of that research." They also referred to the research for the treatment, control and prevention of infectious diseases, which is crucial and require the utilization of pathogenic material as well as laboratory safety and security measures. To provide microbiologists with background information on this topic, Donohue and Atlas informed that "The ASM has also prepared a history of issues and policy related to biological research with select agents and toxins, high containment laboratories, dual use research of concern and gain-of-function (GOF) research. This document is posted on the ASM website at [http://www.asm.org/images/PSAB/History-SelectAgents.pdf]."

- Use of pathogenic *B. anthracis* when non-pathogenic strains would have been appropriate for this experiment
- Inadequate knowledge of the peer-reviewed literature
- Lack of a standard operating procedure or process on inactivation and transfer to cover all procedures done with select agents in the BRRAT laboratory.

What Has CDC Done Since the Incident Occurred

CDC's initial response to the incident focused on ensuring that any potentially exposed staff were assessed and, if appropriate, provided preventive treatment to reduce the risk of illness if exposure had occurred. CDC also ceased operations of the BRRAT laboratory pending investigation, decontaminated potentially affected laboratory spaces, undertook research to refine understanding of potential exposures and optimize preventive treatment, and conducted a review of the event to identify key recommendations.

To evaluate potential risk, research studies were conducted at a CDC laboratory and at an external laboratory to evaluate the extent to which the chemical treatment used by the BRRAT laboratory inactivated *B. anthracis.* Two preparations were evaluated: vegetative cells and a high concentration of *B. anthracis* spores. Results indicated that this treatment was effective at inactivating vegetative cells of *B. anthracis* under the conditions tested. The treatment was also effective at inactivating a high percentage of, but not all *B. anthracis* spores from the concentrated spore preparation.

A moratorium is being put into effect on July 11, 2014, on any biological material leaving any CDC BSL-3 or BSL-4 laboratory in order to allow sufficient time to put adequate improvement measures in place.

What's Next

Since the incident, CDC has put in place multiple steps to reduce the risk of a similar event happening in the future. Key recommendations will address the root causes of this incident and provide redundant safeguards across the agency, these include:

- The BRRAT laboratory has been closed since June 16, 2014, and will remain closed as it relates to work with any select agent until certain specific actions are taken
- Appropriate personnel action will be taken with respect to individuals who contributed to or were in a position to prevent this incident
- Protocols for inactivation and transfer of virulent pathogens throughout CDC laboratories will be reviewed
- CDC will establish a CDC-wide single point of accountability for laboratory safety
- CDC will establish an external advisory committee to provide ongoing advice and direction for laboratory safety
- CDC response to future internal incidents will be improved by rapid establishment of an incident command structure
- Broader implications for the use of select agents, across the United States will be examined.

This was a serious event that should not have happened. Though it now appears that the risk to any individual was either non-existent or very small, the issues raised by this event are important. CDC has concrete actions underway now to change processes that allowed this to happen, and we will do everything possible to prevent a future occurrence such as this in any CDC laboratory, and to apply the lessons learned to other laboratories across the United States.

Background

This report reviews circumstances leading to June 2014 incident in which CDC staff members were potentially exposed to viable *Bacillus anthracis*. The incident occurred after *B. anthracis* extract was transferred from CDC's Bioterrorism Rapid Response and Advanced Technology (BRRAT) biosafety level (BSL) 3 laboratory to BSL-2 laboratories without proper assurance that the extract did not contain viable cells or spores.

This is not the first time an event of this nature has occurred at CDC, nor the first time it occurred from the BRRAT laboratory. At the time of this writing, CDC is aware of four other such incidents in the past decade. In a prior incident in 2006, CDC's BRRAT laboratory transferred vials of anthrax DNA to the Lawrence Livermore National Laboratory (LLNL) and a private laboratory. The BRRAT laboratory believed that they had inactivated the samples, but upon receipt and testing of the samples at LLNL, viable *B. anthracis* was detected. The BRRAT laboratory implemented new quality assurance procedures to ensure non-viability of DNA preparations of select agents and developed policies that require the signature of the laboratory's principal investigator prior to shipping or transferring DNA derived from bacterial select agents. These procedures were not followed in the current incident, which did not specifically involve preparation of DNA for transfer. Also in 2006, DNA preparations shipped from another CDC laboratory were found to contain live *Clostridium botulinum* due to the use of inadequate inactivation procedu-

res. In 2009, newly available test methods showed that a strain of *Brucella*, thought to have been an attenuated vaccine strain and previously shipped to LRN laboratories as early as 2001, was not the vaccine strain. The vaccine strain is not considered to be a select agent, while the strain that was actually shipped is a select agent.

As this report was being finalized, CDC leadership was made aware that earlier this year a culture of low-pathogenic avian influenza was unintentionally cross-contaminated at a CDC influenza laboratory with a highly pathogenic H5N1 strain of influenza and shipped to a BSL-3, select-agent laboratory operated by the United States Department of Agriculture (USDA). The CDC influenza laboratory where this incident occurred is now closed and will not reopen until adequate improvements are put in place. Although CDC is continuing to investigate and review this matter, Attachment A provides current information on the incident and the agency's response.

Effective, validated inactivation protocols for *B. anthracis* have been published. Cultures of *B. anthracis* cells and spores can be completely inactivated through established protocols using heat (e.g., boiling for 10 minutes or autoclaving for 15 minutes), irradiation (1 million rad), or various chemical treatments (e.g., hydrogen peroxide, peracetic acid, formalin, or gaseous ethylene oxide). In general, longer treatment times and/or higher concentrations are required for inactivation of spores compared to inactivation of viable cells. Solutions can also be sterilized by filtration, through a 0.1 micron filter, to remove viable cells and spores.

Space decontamination can be achieved through one of two approved liquid decontamination methods and one vapor method. A solution of freshly made dilution of household bleach (10% bleach by volume), pH adjusted to 7.0 with acetic acid, is recognized by the Environmental Protection Agency (EPA) to kill *B. anthracis* spores with a minimum contact time of 10 minutes. The EPA also registered the use of Spor-Klenz® (STERIS®) as a sterilant, as a 1:99 water dilution of the concentration is effective as a sporocide with a minimum contact time of 30 minutes. Vapor phase hydrogen peroxide is also available at CDC as a room disinfectant.

Laboratories

CDC laboratories conduct research that is critical to better detect, respond to, and prevent disease and bioterrorism. Research done in CDC laboratories helps identify better ways to detect these infectious agents rapidly. The Laboratory Response Network (LRN) is a network of laboratories that can respond to biological and chemical threats and other public health emergencies. It includes state and local public health, veterinary, military, and international labs. The BRRAT laboratory provides technical and scientific support for the approximately 150 laboratories in the LRN. The BRRAT laboratory contains both BSL-3 and BSL-2 labs and was established in 1999 in accordance with Presidential Decision Directive 39, which outlined national anti-terrorism policies and assigned specific missions to federal departments and agencies (http://www.bt.cdc.gov/lrn/). The BRRAT laboratory provides quality assurance for the specialized reagents used in the LRN and has performed studies with the goal of improving the performance and reliability of tests used to detect biological threat agents. *Bacillus anthracis* is of particular concern because it can and has been used as a weapon.

Two CDC laboratories received the extracts prepared by the BRATT laboratory BSL-3 laboratory: the Bacterial Special Pathogens Branch laboratory (BSPB laboratory); and the Biotechnology Core Facility Branch (BCFB laboratory).

Methods Used in Reviewing this Incident

A CDC team of scientists and leaders interviewed laboratory scientists involved directly with the incident and others who had specific knowledge of the incident and of immediate response activities. Each interview consisted of a standardized set of questions, as well as specific questions based on an individual's role and responsibilities. Standard operating procedures (SOPs), protocols, and training records were also reviewed.

Description of the Event

The BRRAT laboratory was evaluating matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which can identify bacteria by bacterial protein "fingerprints." It is faster and less expensive than conventional species-identification methods, which require culture of organisms on selective bacterial media or extraction and characterization of bacterial nucleic acids. The project was a collaboration among the BRRAT, BSPB, and BCFB laboratories. The researchers intended to use the data collected to submit a joint proposal to CDC's Office of Public Health Preparedness and Response to fund further evaluation of the MALDI-TOF method because MALDI-TOF is increasingly being used by clinical and hospital laboratories for infectious disease diagnostics.

On June 2, 2014, the BRRAT laboratory supervisor contacted a subject matter expert who had successfully used this technology to identify three pathogenic species of *Brucella*.

In response to the BRRAT laboratory supervisor's request for assistance and advice, a BSPB laboratory supervisor offered to share the methodology, results, and inactivated bacterial preparations used by the BSPB laboratory in their work with *Brucella*. The BSPB laboratory had modified the MALDI-TOF equipment manufacturer's sample preparation protocol to optimize the results for bacterial protein sample extractions of *Brucella*. In this extraction procedure, each organism is treated with ethanol, then with 70% formic acid for 10 minutes, followed by the addition of 100% acetonitrile, and then is incubated at room temperature. The method used by the BSPB laboratory also incorporated a sterility check of the extract after 10 minutes of incubation in the extraction solution. Specifically, an aliquot of the extract was spread on an agar plate, incubated for 48 hours, and then examined for growth. If no growth was visible, the extract was considered to be sterile and could be safely transferred from the BSL-3 laboratory to a BSL-2 laboratory for processing for use in the MALDI-TOF equipment.

The BSPB laboratory protocol did not call for filtration of the bacterial extract prior to transfer from the BSL-3 laboratory because it had been determined that the extraction procedure inactivated the three species of *Brucella* tested. It is important to note that, unlike *B. anthracis*, *Brucella* does not form spores. Bacterial spores are relatively resistant to harsh conditions, such as the chemicals used in this extraction procedure, and are more difficult to kill than vegetative cells. As a result, additional procedures (e.g., filtration) can be used when working with spore-forming bacteria, such as *B. anthracis,* to ensure specimens are rendered non-viable.

The BRRAT laboratory supervisor instructed a laboratory scientist to obtain the written protocol for sample preparation from BSPB laboratory. The BSPB laboratory provided a sample preparation protocol, which did not include a viability SOP. The supervisor requested that virulent strains of eight select agents, including *B. anthracis,* be used for the initial experiment. On June 5, 2014, the laboratory scientist followed the modified protocol to prepare eight individual organism extracts for use in the MALDI-TOF. Another scientist in the BRRAT laboratory raised the question of whether filtration of the extracts might affect the MALDI-TOF results. To answer this question the laboratory scientist split each extract into two aliquots and filtered one aliquot through a 0.1 micron filter. After a 10 minute incubation period, filtered and unfiltered extracts were then plated onto agar and incubated for 24 hours to check the extracts for sterility. The decision to incubate for 24 hours, rather than 48 hours (as recommended by the BSPB staff member) was made by the first laboratory scientist based on the individual's own understanding of information conveyed by the laboratory scientist in the BSPB laboratory during a telephone discussion of the protocol.

All work was performed in a biological safety cabinet in the BRRAT BSL-3 laboratory with both BRRAT laboratory scientists present. The first laboratory scientist was primarily involved in performing the extraction, and the second was there to observe and learn the procedure. Both were jointly involved in filtering material, plating onto media, and reading sterility plates at 24 hours. After 24 hours of incubation, they observed no growth on any of the 16 sterility plates that had been prepared after 10 minutes of formic acid treatment. The first laboratory scientist planned to autoclave the plates, then discard them; however, the individual had difficulty opening the autoclave door. As a result, the plates were returned to the incubator and left for 7 additional days.

The first laboratory scientist moved the extracts from the BRRAT laboratory BSL-3 lab to an adjoining BSL-2 laboratory that is also part of the BRRAT laboratory. At this point, the protein extracts had been held in the formic acid/acetonitrile solution for 24 hours. The first laboratory scientist then continued with the process of preparing the material for analysis by MALDI-TOF, and then moved preparations or aliquots of the protein extracts made from the BRRAT's BSL-2 laboratory to the BSPB and BCFB laboratories on three separate days: June 6, June 11, and/or June 12, 2014.

On June 13, 2014, the second BRRAT laboratory scientist removed the sterility testing plates after 8 days in the BSL-3 incubator for autoclaving and disposal and discovered growth on the sterility plate that had been plated with unfiltered *B. anthracis*. The growth was confirmed as *B. anthracis* by real-time polymerase chain reaction using the LRN *B. anthracis* identification assays. It is not known at what point after the initial 24 hour incubation period that growth occurred. If the plates had been autoclaved after 24 hours, as planned, the event would not have been discovered.

The incident was immediately reported to the CDC Select Agent Program Responsible Official within CDC's Environment, Safety and Health Compliance Office (ESHCO) and DSAT.

CDC personnel decontaminated the affected rooms using the liquid decontamination methods described above (see Background). Laboratory floors, benchtops, equipment, and other affected areas (e.g., room door handles) were decontaminated as part of this process. Two potentially affected refrigerators were moved to a secure BSL-3 facility and decontaminated using vapor phase hydrogen peroxide. Rooms will remain closed until the procedures have been validated as EPA compliant by an external safety expert.

After the incident was discovered, two laboratory studies were undertaken to determine if the formic acid and acetonitrile treatment was effective at inactivating laboratory specimens of *B. anthracis*: one at CDC and one at an independent LRN laboratory at the Michigan Department of Community Health (MDCH). The CDC study evaluated the effect of treatment exposure times of 10 minutes in formic acid and after 6 hours and 24 hours in formic acid/acetonitrile on *B. anthracis* vegetative cells. In addition, the CDC study evaluated treatment exposure times of 10 minutes in formic acid and 24 hours in formic acid/acetonitrile using high-concentrations of *B. anthracis* spores. Cultures from treated cells and spores were monitored daily for viability for up to 8 days post-treatment. The MDCH study independently evaluated the efficacy of the formic acid/acetonitrile treatment on *B. anthracis* vegetative cells. This study used samples that were taken at three different time points: immediately on addition of the formic acid and subsequently at 1 hour and 24 hours post-treatment. The MDCH cultures were monitored for up to 8 days for viability.

Findings from both the CDC internal study and the MDCH indicate that the formic acid and formic acid/acetonitrile treatment were effective at inactivating vegetative cells of *B. anthracis*. No viable material was recovered from formic acid and formic acid/acetonitrile treated cells. These findings were consistent for the 8-day study duration. The formic acid and formic acid/acetonitrile treatments were effective at inactivating a high percentage, but not all, *B. anthracis* spores. From a starting suspension of 50,000 *B. anthracis* spores (500,000 per milliliter), which had been treated for 24 hours with the extraction process, there were a total of four colony forming units of growth in the 8-day study period.

Based on review of all aspects of the incident, it appears that while exposure of staff to viable *B. anthracis* was not impossible, it is extremely unlikely that this occurred. All or the great majority of *B. anthracis* cells and spores in the sample would have been inactivated by the 24-hour treatment (versus the 10 minute sample which grew anthrax at some point between day 2 and day 8 of incubation).

Findings

Incident-related Findings

The overriding factor contributing to this incident was the lack of an approved, written study plan reviewed by CDC senior staff, such as laboratory, branch, or division scientific leadership, to ensure that the research design was appropriate and met all laboratory safety requirements. The first BRRAT laboratory scientist was trained to work in the BSL-3 environment, including training in pathogen-specific procedures for the work normally performed. However, the individual had not performed this specific procedure with pathogenic select agents (the procedure was new to the laboratory) and should not have been instructed to proceed without submitting a complete protocol for review and approval. Further, a written protocol to certify the sterility of material to be transferred to BSL-2 laboratories was not in place, and the BSL-2 laboratories did not have an SOP that required receipt of written certification of non-viability for transfers prior to acceptance of microbiologic material. There was also inadequate supervisory oversight of a relatively new laboratory scientist performing a new experiment with virulent strains.

The first laboratory scientist also assumed that the protocol was appropriate for *B. anthracis*. It appears that there was incomplete communication between the two BRRAT laboratory scientists and the BSPB laboratory scientist about what was planned by the BRRAT laboratory and what had previously been done by the BSPB laboratory. The procedure used by the BSPB laboratory for *Brucella* species did not include a filtration step because the BSPB laboratory determined it was not necessary for extracts of *Brucella* based on the sterility testing they had done on extract material of three species of *Brucella*. Since *B. anthracis* forms spores that are more resistant to inactivation by chemicals than vegetative cells, the BRRAT laboratory scientist's assumption that the same treatment would apply to *B. anthracis* was incorrect.

The BRRAT laboratory scientist did not plan to filter extracts because it was not part of the BSPB laboratory protocol. The BRRAT laboratory scientist was aware that all DNA preparations of *B. anthracis* were filtered before leaving the BSL-3 laboratory, but assumed that it was not necessary for MALDI-TOF preparations because a filtration step was not included in the protocol. The BRRAT laboratory scientist had no previous experience transferring select agent-derived materials, other than transferring DNA preparations, from BSL-3 to BSL-2 laboratories. The BRRAT laboratory's SOP for assuring sterility was specific for DNA preparations, and SOPs for other materials do not appear to have been in place. The SOP for DNA preparations (with which the first BRRAT laboratory scientist was familiar) indicated that sterility check plates for *B. anthracis* should be held for 24-48 hours.

It is not clear that waiting 48 hours rather than 24 hours to transfer the extracts would have prevented this incident. The bacterial cells or spores were damaged by the extraction pro-

cedure, and the direct plating of the extract carried over chemicals which could have inhibited growth. Acceptable practice would have been to utilize validated methods to confirm sterility.

The following actions contributed to the incident:

1. **Use of unapproved sterilization techniques**: Staff in the BRRAT laboratory used sample preparation techniques for protein extraction from the manufacturer of the MALDI-TOF equipment, modified by the BSPB laboratory for non-spore forming bacteria (*Brucella* species) to sterilize *B. anthracis*, a spore-forming bacterium. A laboratory scientist modified the methods from the BSPB laboratory to include comparing filtration versus non-filtration in preparing 16 plates (half filtered and half not filtered). This modification was done to assess any effects on the MALDI-TOF results, not to assure sterility. The incubation period was also shortened from 48 hours to 24 hours.

2. **Transfer of material not confirmed to be inactive**: After 24 hours without observing growth on the sterility plates, the BRRAT laboratory scientist moved the extracts from the BRRAT laboratory BSL-3 laboratory to an adjoining BSL-2 laboratory, and then continued with the process of preparing the material for analysis by MALDI-TOF. The BRRAT laboratory scientist then moved the extracted materials from the BRRAT laboratory's BSL-2 laboratory to the BCFB and BSPB laboratories on three separate days: June 6, June 11, and/or June 12, 2014. There is a lack of written procedures which had been validated to reliably ensure that organisms were no longer viable prior to removing microbiological material from BSL-3 containment.

3. **Use of pathogenic B. anthracis when non-pathogenic strains would have been appropriate for this experiment:** The BRRAT laboratory supervisor instructed the laboratory scientist to use virulent strains because of the possibility that avirulent strains might not yield the same MALDI-TOF profile. However, the instrument manufacturer states that the system identifies bacteria to only the species level and would not distinguish strains of the same species. The use of avirulent strains to develop protocols would have been appropriate, particularly when conducting a pilot study.

4. **Inadequate knowledge of the peer-reviewed literature by the BRRAT laboratory supervisor and scientist who performed the extraction**: A review of the literature would have found that filtration has been recommended for inactivation of *B. anthracis*. There are at least two peer-reviewed publications on preparation methods for MALDI-TOF work with pathogenic bacteria, including *B. anthracis* (Drevinek et al. Letters in Applied Microbiology 2012;55:40-46; and Lasch, et al. Analytical Chemistry 2008;80:2026-2034). While the chemicals used to process the samples differ in the two publications, both required filtration of *B. anthracis* material with a 0.1 micron filter to remove spores. Drevinek et al. (2012) concluded that the formic acid method (as used by the BRRAT laboratory) did not sterilize B. *anthracis*; they also used centrifugal filtration to remove viable particles (including spores) from *B. anthracis* preparations.

5. **Lack of a standard operating procedure or process to document inactivation in writing in the BRRAT laboratory**: With correct SOPs in place that are adhered to by staff, microbiological material would have been successfully inactivated prior to transfer to a lower containment laboratory (either intra- or inter-facility) and a record of non-viability would have been provided to the receiving laboratory; also, a written record of non-viability would have been provided prior to receipt and utilization of the microbiological materials in the BSL-2 laboratories.

Response-related Findings

On June 13, 2014, two CDC staff members went to the emergency department at Emory University where they were assessed; neither presented with symptoms related to anthrax. Staff were assessed based on their risk of potential exposure that could lead to inhalational anthrax. The number of potentially exposed staff evolved as understanding of the laboratory events unfolded. Additional potentially exposed individuals were identified through supervisor discussions with individuals believed to have handled or been in proximity to the *B. anthracis* material. The process of identification was slowed by multiple factors, including the evolving nature of understanding of the event. Technology 10 resources such as card key readers and security video were utilized to expand the pool of potential exposures, but this was not an immediate step in the response. Even with the use of available data, several factors made the identification process difficult, including the practice of authorized staff piggy backing (obtaining entrance to a secured area by following a colleague rather than by having all individuals swipe their own card key as should

be done) and incomplete or inaccurate information collected from laboratory scientists reporting their path of travel with the material between labs. Protocols were not in place for the rapid identification of potentially exposed staff, possibly delaying the use of available data sources including card key readers, visitor logs, and security video logs.

Immediate and comprehensive actions were taken to identify the potentially affected laboratory rooms as well as the individuals that were or may have been in, or traveled through, these areas during the time period of possible exposure. After ascertaining the precise events that took place in the laboratories and characterizing people's possible exposure was difficult and evolving, there were serious reservations on the part of some staff members of the affected laboratories and others about broad communication until sufficient information was gathered and verified. In retrospect, it is clear that broad communications should have occurred earlier in the process, even if more complete information was not yet available. CDC scientists who worked near the impacted laboratories commented that they first learned of the event by witnessing CDC closing and/or decontaminating laboratories rather than through direct communication regarding the ongoing event. In addition, there were inconsistencies in the decontamination practices used after the incident, which made it difficult to ensure proper methods were used. Individuals also reported the CDC clinic was overwhelmed at times during the response.

The nature of this incident required involvement of many parties from across CDC. While the roles of the responders were generally clear and appropriate actions were taken, there was no clear overall lead for the incident in the first week. This resulted in uncertainty regarding who was responsible for making decisions and taking action.

As of July 10, 2014, no staff members are believed to have become ill with anthrax.

Actions Already Underway and Plans for the Future

A moratorium was initiated July 11, 2014, on any biological material leaving any CDC BSL-3 or BSL-4 laboratory in order to allow sufficient time to put adequate improvement measures in place. In addition, CDC has already begun steps to protect staff and prevent similar incidents in the future. Key actions are planned to address the root causes of this incident. The recommendations focus on specific actions that provide redundant safeguards across the agency.

These actions and recommendations relate to

- The BRRAT laboratory
- Inactivation and transfer procedures of virulent pathogens throughout CDC laboratories
- Broader improvements in biosafety in laboratories throughout CDC
- CDC response to internal incidents
- Broader implications for the use of select agents, including for CDC's regulatory functions through CDC's Division of Select Agents and Toxins.

The BRRAT Laboratory

1. The laboratory has been closed since June 16, 2014, and will remain closed as it relates to work with any select agent. This action was reinforced by USDA's Animal and Plant Health Inspection Services (APHIS). Laboratory scientists do not have access to select agents, which have been placed in storage-only mode. The unit will remain closed with respect to select agents until the following is completed:
   a. An assessment and appropriate follow-up actions for all BRRAT laboratory staff to determine level of skills, training, supervision, knowledge, and expertise at all levels of the organization
   b. The establishment of clear, proven procedures that have been communicated to all staff for inactivation and non-viability testing of all types of materials that may be produced by the laboratories (i.e., not limited to nucleic acid preparations from one specific laboratory) and documentation of these processes
   c. Resolution of all findings included in this report and in the APHIS investigation report

2. Appropriate personnel action will be taken with respect to individuals who contributed to or were in a position to prevent this incident.

Inactivation and Transfer Procedures of Virulent Pathogens throughout CDC Laboratories

3. All inactivation procedures for laboratories working with select agents and other dangerous pathogens are being carefully reviewed and will be updated as needed. This includes, but is not limited to, any inactivation performed in conjunction with MALDI-TOF testing. CDC will notify the MALDI-TOF manufacturer and the Food and Drug Administration (FDA) of this event and encourage the de-

velopment of informational materials that are clearer regarding appropriate inactivation procedures for all types of pathogens. All CDC laboratories that handle select agents and other dangerous pathogens will be confirmed to have written, validated, and verified procedures to assure materials are non-viable before being removed from containment and to assure the provision of written documentation of non-viability, including the method used, for intra- and inter-facility transfers. These procedures will include requirements that all transferring laboratories confirm non-viability by proven, effective methods before material leaves the containment laboratory and provide documentation to accompany the transfer and that the receiving laboratory confirm the materials are not viable. When new procedures, techniques, or manufacturer methods are being considered, they must first be reviewed and evaluated through a formal process to assess their risk and incorporate them into standard CDC policies, procedures, and practices prior to implementation.

Laboratories across CDC

4. CDC will establish a lead laboratory science position to be the CDC-wide single point of accountability for laboratory safety. The creation of a single point of accountability does not reduce the responsibility of people at every level of the organization, including center, division, and branch directors, chiefs, supervisors, and all laboratory scientists to strengthen the culture of safety. This position will:
   a. Establish and enforce agency-wide policies that require formal review and approval of new select agent research or program protocols and provide oversight for ongoing research and program projects (e.g., yearly reviews).
   b. Create effective and redundant systems and controls for protocols and procedures including, but not limited to, inactivation and access to laboratories (e.g., "piggybacking" and visitor access).
   c. Ensure adherence to laboratory quality and safety protocols (e.g., quality assurance that biological material is non-viable before it is shipped from CDC select agent laboratories). These protocols will be transferred to new staff whenever there is a turnover in select agent laboratories, especially when there is a new principal investigator.
   d. Review and monitor the implementation of training policies and procedures for new and existing staff.

5. Use an approach that identifies the points in any project where potential mistakes would have the most serious consequences that provides specific actions to avoid these mistakes. Examples of these critical points and associated preventive actions include requiring protocols to be reviewed by supervisors before they are implemented, having standard and clear procedures to inactivate infectious agents and specify how they will be transferred to other labs, having formal incident response plans in place, controlling laboratory access, and instituting regular review of laboratory processes to ensure proper safety, quality management, and compliances with Select Agent Regulations.
   a. Identify ways to decrease the risk of an event such as this happening again, which may include fewer laboratories working with select agents and/or a decrease in the number of pathogenic strains being studied and/or a decrease in the number of staff members working with these agents.
   b. Promote the use of non-pathogenic organisms in research and training activities, whenever possible.
   c. Accelerate the ongoing implementation of laboratory quality management systems (QMS) throughout CDC laboratories. Over the past 5 years, CDC has begun implementing a QMS for infectious disease laboratories which includes document controls such as protocol archives and approval records as an integral part. Initial adoption of QMS has focused on the laboratories with clinical diagnostic responsibilities and has greatly enhanced their safety and efficiency. Expansion into nonclinical laboratories has been ongoing and will now be accelerated as a high priority, with QMS becoming an integral part of CDC laboratory management practice.

6. CDC will establish an external advisory committee to provide ongoing advice and direction for laboratory quality and safety. It is likely this advisory committee will be established under the Federal Advisory Committee Act (FACA).

Response Efforts

7. CDC will initiate an incident command structure early in any response to an incident at CDC when an event is suspected that the incident is significant or not well understood. CDC may also leverage the assets of CDC's Emergency Operations Center to help coordinate the event response under the incident commander. This does not necessarily mean activating the EOC for such a purpose, but use of the EOC facility, staff, tools, and other resources as well as coordination within CDC offices could be beneficial. Under this structure, CDC can ensure proactive and

frequent communication with staff, media, and the public. This structure will also allow for quick access to CDC staff with unique expertise to provide surge capacity (including nurses and physicians to staff the CDC clinic), as needed.

Broader Implications for the Use of Select Agents

8. Lessons based on this incident that will be considered for broader implications. CDC's DSAT program will incorporate findings and recommendations into nationwide regulatory activities to provide stronger safeguards for laboratories across the United States. For example, in its review of biosafety plans with regulated entities, DSAT will emphasize the importance of having proven inactivation protocols and utilizing testing for inactivated preparations prior to distribution.

Conclusion

Potential exposure of CDC laboratory scientists to anthrax occurred as a result of a series of failures of one laboratory (the CDC BRRAT laboratory) to ensure that *B. anthracis* specimens had been inactivated before transferring them to other laboratories at CDC. This same laboratory had inadvertently transferred viable *B. anthracis* on a previous occasion in 2006. Review of the procedures and practices that allowed this event to occur identified: failures of policy, training, scientific knowledge, supervision, and judgment on the part of this laboratory. In addition, there was a lack of adequate agency-wide policies and procedures to ensure biosafety, both for decontamination of select agents and other virulent organisms as well as for biosafety more broadly. Further, biosafety policies and procedures adopted in the past were not always adhered to in the present. Response to the incident should have been better organized from the outset.

Review of the incident suggests that it is highly unlikely, but not impossible, that staff members were exposed to viable *B. anthracis*. None of the potentially exposed workers has become ill with anthrax. Nonetheless, this was a serious and unacceptable incident which should never have happened. A moratorium is being put into effect on July 11, 2014, on any biological material leaving any CDC BSL-3 or BSL-4 laboratory in order to allow sufficient time to put adequate improvement measures in place. Five key steps are being taken immediately: suspension of activities of this individual laboratory pending full review and remediation of all procedures and practices; agency-wide verification of adequate inactivation procedures; strengthening of biosafety agency-wide with appointment of a single point of accountability and through an external group of experts to review and advise CDC; improvement of management of internal incidents with use of an incident management system; and use of lessons learned from this incident to strengthen CDC's regulatory function with regard to select agents.

Given both the critical nature of investigations to enable CDC to improve our ability to detect and respond to naturally occurring and man-made events with select agents and the paramount responsibility of ensuring the safety of CDC staff members when they do this work, CDC leadership, including the CDC Director, will track the rapid and effective implementation of these plans.