

Report on the Potential Exposure to Anthrax

Centers for Disease Control and
Prevention

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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
- 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 procedures. 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

Appendices B and C provide a timeline of major events.

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 procedure, 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

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 development 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.

Appendix A – Summary of the Inadvertent Shipment of an Influenza Virus H5N1-containing Laboratory Specimen

On July 9, 2014, CDC's select agent office and agency leadership were notified that the a low-pathogenic avian influenza sample was inadvertently cross-contaminated with a select agent, the highly pathogenic H5N1 influenza virus, before being shipped from an influenza laboratory to the US Department of Agriculture Southeast Poultry Research Laboratories (SEPRL). The H5N1 influenza was a contaminant of a low-pathogenic avian influenza virus specimen that is not a select agent. Since the influenza laboratory was unaware of the contamination, appropriate select agent transfer procedures were not followed. Because the materials were handled during shipping as 'category B' (standard shipping procedures for infectious agents) and all laboratory work in both institutions was carried out in enhanced BSL-3 facilities, there does not appear to be any safety risk posed by this incident.

Investigation of the incident thus far found that contamination of the low-pathogenic influenza virus specimen with the highly pathogenic H5N1 influenza virus occurred during laboratory work at the CDC, leading to samples being shipped without the appropriate level of permitting, notifications, or safety precautions. All work with live virus at SEPRL was conducted in their APHIS select agent approved BSL3 facilities. The ongoing investigation also has revealed unacceptable delays in reporting of the inadvertent shipment of the select agent, which was shipped on March 13, 2014, informed to CDC by SEPRL to have been contaminated on May 23, 2014, and confirmed by CDC to have been contaminated in the following days. The H5N1-containing contaminated specimens at both SEPRL and CDC have been or will be destroyed. Confirmation of the contaminated specimens was conducted without notification of the supervisory chain of command including division, center, and CDC leadership.

In response to this incident, and in conjunction with the response to the June 2014 incident of potential exposure to anthrax, CDC has initiated the following steps:

1. Established a high-level working group, reporting to the CDC Director, to, among other duties, accelerate improvements in laboratory safety; review and approve, on a laboratory-by-laboratory basis, resumed transfer of biological materials outside of BSL-3 and BSL-4 laboratories; and serve as the transition group for the single point of accountability on laboratory safety.
2. Begun the process of establishing an external advisory group for laboratory safety.
3. Initiated an investigation to determine root causes that led to contamination of another avian influenza virus by the H5N1 virus.
4. Reported the incident through the proper channels to the select agent oversight body, APHIS.
5. Established a review group, under the direction of CDC's Associate Director for Science, to look at the systems, procedures, and personnel issues leading to this event and means of preventing similar events in the future. This review will be done in conjunction with the internal investigation and in coordination with the working group.
6. Undertaking appropriate personnel action expeditiously.

Beyond these specific steps, the CDC-wide moratorium on any biological material leaving any CDC BSL-3 or BSL-4 laboratory (with effect from July 11, 2014) applies to this laboratory.

Appendix B – Timeline of Major Events

Note: Data reported below were current as of July 10, 2014.

June 5 (Thursday):

- A laboratory scientist in the Bioterrorism Rapid Response and Advanced Technology (BRRAT) laboratory prepared extracts from a panel of select agents including *B. anthracis* (Ames strain) for analysis using mass spectroscopy while in the BRRAT laboratory biosafety level 3 (BSL-3). After performing the extractions, the BRRAT laboratory worker took samples of the extracts for sterility testing after 10 minutes and continued treatment for a total 24-hour incubation.

June 6 (Friday):

- After 24 hours all plates of the extracts for sterility testing were examined by two laboratory scientists and no growth was observed. Two of these plates had *B. anthracis* (Ames strain).
- Extracts were then moved from the BRRAT laboratory BSL-3 to the BRRAT laboratory BSL-2 for further processing and preparation of a steel MALDI-TOF plate. This processing is regarded as a potential for aerosolization of the material.
- The dried MALDI-TOF plate was placed inside a plastic MALDI-TOF plate holder with a closed lid and then placed in a zip locked bag and delivered to the Biotechnology Core Facility Branch (BCFB), a BSL-2 laboratory in another building, and then taken for mass spectroscopy analysis in the BCFB laboratory.
- A laboratory scientist in BCFB laboratory noticed small flakes of material on the MALDI-TOF plate and took it to a chemical hood in that room and used a nitrogen air stream to blow off the flakes. This is regarded as a potential for aerosolization of the material.
- The MALDI-TOF plate was stored in its plastic container, with a closed lid, on the bench in BCFB Laboratory at room temperature.

June 7 - 8 (Saturday & Sunday):

- No activity related to this incident occurred. The plate was stored in its plastic container with a closed lid, on a lab bench BCFB laboratory.

June 9 (Monday):

- No activity related to this incident occurred. Plate was stored in its plastic container with a closed lid, on a laboratory bench BCFB laboratory.

June 10 (Tuesday):

- No activity related to this incident occurred. Plate was stored in its plastic container with a closed lid, on a laboratory bench BCFB laboratory.

June 11 (Wednesday):

- A laboratory scientist from the BRRAT laboratory brought a closed zip-locked bag with eight closed tubes, each tube containing 6 microliter aliquots of the extracts, from the BRRAT laboratory BSL-2 to the BCFB laboratory where it was stored in a refrigerator at +4°C. One of the tubes included an unfiltered extract prepared from *B. anthracis* on June 5, 2014.

June 12 (Thursday):

- A laboratory scientist in BCFB laboratory removed the samples from the refrigerator in the Linear Equipment Room (LER) near BCFB laboratory, and took them to another room in BCFB laboratory to be prepared under a biological hood for testing on the MALDI-TOF mass spectrometer.
- The MALDI-TOF plate was then taken to a third BCFB laboratory room, for mass spectroscopy analysis. No flaking was observed.
- A laboratory scientist in BRRAT laboratory repeated the MALDI-TOF preparation process in BRRAT BSL-2 laboratory on an open bench using extracted materials prepared on June 5, including the unfiltered *B. anthracis* extract. Preparation of the MALDI-TOF plate included vortexing the extract material, which is a potential for aerosolization of the material. The vortexing is high-powered spinning of the liquid to prepare for further testing. The dried MALDI-TOF plate was placed inside a plastic MALDI-TOF plate holder with a closed lid and then was placed in zip locked bag and delivered to the Bacterial Special Pathogens Branch (BSPB) laboratory for mass spectroscopy analysis.

June 13 (Friday):

- At 5:00 PM, a laboratory scientist in BRRAT laboratory BSL-3 was removing sterility plates from the incubator and observed growth on one plate labeled as representing *B. anthracis* (Ames strain). The identity of the organism was confirmed using real-time polymerase chain reaction (PCR) and Laboratory Response Network (LRN) approved assays. Typically, real time PCR takes ~2hours once DNA is prepared. DNA extraction can take ~45 minutes to 1 hour.
- At 5:15 PM, per the BRRAT Laboratory Incident Response Plan, the second laboratory scientist contacted the Select Agent Responsible Official (RO), who is the agency's identified official overseeing the Centers for Disease Control and Prevention's (CDC) Select Agent Compliance Program.
- At 7:00 PM, all materials distributed to other labs had been collected by BRRAT laboratory personnel and returned to BSL-3 containment. Two laboratory scientists (one from the BRRAT laboratory and one from the BCFB laboratory) were referred to Emory University Hospital Emergency Room and placed on antibiotics. Swabs were taken of affected work areas in the BRRAT laboratory and BCFB laboratory, and the work areas were then decontaminated by lab personnel.
- At 7:28PM RO contacted the BRRAT laboratory supervisor to request a status on the written summary.
- At 8:16 PM, laboratory management sent initial report of incident to the RO to support development of the content that will be entered on the Form 3 and reported to the Division of Select Agent and Toxins (DSAT)

- At 8:41 PM, RO officially notified DSAT that a release of *B. anthracis* has occurred. RO began completion of Form 3.
- At 11:07PM, CDC senior leadership were notified of incident.
- DSAT provided the United States Department of Agriculture's (USDA) Animal Plant Health Inspection Service (APHIS) with an informal notification, by phone.
- Supervisor discussions occurred to immediately identify staff who may have had any potential exposure to *B. anthracis*.
- Affected work areas in BRRAT and BCFB laboratories were decontaminated with 10% bleach for longer than 10 minutes followed by 70% ethanol.

June 14 (Saturday):

- Data were reviewed to better understand the exposure pathway and identify staff who should be assessed and placed on post-exposure prophylaxis (PEP) with antibiotics and vaccine. Initially 10 staff were identified as possibly being exposed.
- Mass spectrometers in BCFB laboratory and BSPB laboratory were shut down and will remain shut down until CDC receives advice from the manufacturer.

June 15 (Sunday):

- Further discussion occurred regarding the exposure pathway and methods to identify staff to assess and recommend for PEP.

June 16 (Monday):

- BRRAT laboratory closed.
- Further discussion occurred on the development of a risk-assessment matrix to better determine who might have been potentially exposed.
- By late Monday, staff identified the following staff as being potentially exposed to *B. anthracis*:
 - BRRAT Laboratory: 9 staff
 - BCFB Laboratory: room 1, 2 staff; room 2, 2 staff; room 3: 8 staff
 - BSPB Laboratory: 1 staff
- Distribution of antibiotics and vaccine to potentially exposed staff began.
- BCFB laboratory, room 3, was decontaminated and closed. Rooms 1 and 2 were decontaminated and deemed operational, but remain closed.
- Environmental sampling plan following LRN protocols was determined for BCFB laboratory, room 3, and BSPB laboratory.
- Exterior of the mass spectrometer in BSPB laboratory was decontaminated.
- Subject matter experts determine that staff should be placed into two exposure categories-- staff who were potentially exposed and staff who were not potentially exposed. It was not possible to gauge the level of exposure based on criteria other than presence in a room.
- It was determined that 25 individuals were potentially exposed. These staff were identified by self-referrals or supervisors/project investigators who have projects in the affected laboratories and asked to contact the clinic.

June 17 (Tuesday):

- RO formally requests Personnel Suitability Board review of all BRRAT Laboratory Tier 1 staff.
- Card readers were reviewed to assist in identifying staff who may have been in the areas where potential exposure may have occurred.
- 35 staff now determined as potentially exposed through the use of card key logs to identify the additional staff; potentially exposed staff were contacted to schedule a clinic visit.

June 18 (Wednesday):

- Environmental sampling plan developed and swabs taken in BSPB laboratory (3 locations) and in BCFB laboratory (14 locations). Plates will be observed for growth for 8 days. Following the sampling all areas were decontaminated following EPA established protocols.
- Card key logs were used to identify the additional staff, and they were contacted to schedule a clinic visit. CDC initiated a review of visitor logs: 67 individuals determined as potentially exposed based on the additional sources.

June 19 (Thursday):

- In BRRAT laboratory BSL2, floors are sprayed with Spor-Klenz[®], but due to concerns about the material damaging the equipment, lab staff requested they be provided the solution (which is stable for 7 days) and offered to clean the benches and equipment themselves.
- Personnel Suitability Board met and began to assess staff training compliance.
- On request by the CDC Director, the Office of the Associate Director for Science began internal review of the events that took place in the BRRAT laboratory on June 5 that evolved into a possible *B. anthracis* exposure event.
- DSAT briefs APHIS on the incident based upon preliminary information available.
- Form 3 completed and submitted by the RO to DSAT.

June 20 (Friday):

- Clinic hours expanded to accommodate additional staff visits to the clinic.
- DSAT provides APHIS with the Form 3.
- CDC notified of official APHIS inspection to begin on June 23 with support from DSAT inspectors.

June 21 - 22 (Saturday & Sunday)

- Formation of systematic Response Team, with workgroups established to focus on Clinical Care, Environmental Sampling, Epidemiology, Laboratory, Policy, and Communication.
- CDC laboratory studies initiated to determine if the procedures used in the BSL-3 laboratory inactivated the *B. anthracis*.

June 23 (Monday):

- Response Team convenes using the CDC Emergency Operations Center platform for logistics support.
- APHIS inspectors submit requests for information to begin paper reviews.

June 24 (Tuesday):

- In-depth risk assessment started and follow up of CDC staff and contractors potentially exposed to *B. anthracis*.
- APHIS kickoff meeting takes place.
- Additional visitors (three) self-identified as possibly exposed. They are referred to clinic for assessment. All subsequently consulted with the clinic and provided medication.

June 25 (Wednesday):

- Visitor log review completed. No new potentially exposed individuals identified to date.
- External collaborator laboratory study initiated to determine if the procedures used in the BSL-3 laboratory inactivated the *B. anthracis*.

June 26 (Thursday):

- All environmental samples taken have been observed daily and reported each time as negative after 8 days of incubation, which concludes the testing process for these samples.

June 28 - 29 (Saturday & Sunday):

- Environmental data reviewed to help inform revision of risk assessment. Results from the CDC laboratory studies and findings to date from the external collaborator laboratory study indicate that it is unlikely that viable bacteria left the BSL-3 lab.

June 30 (Monday)

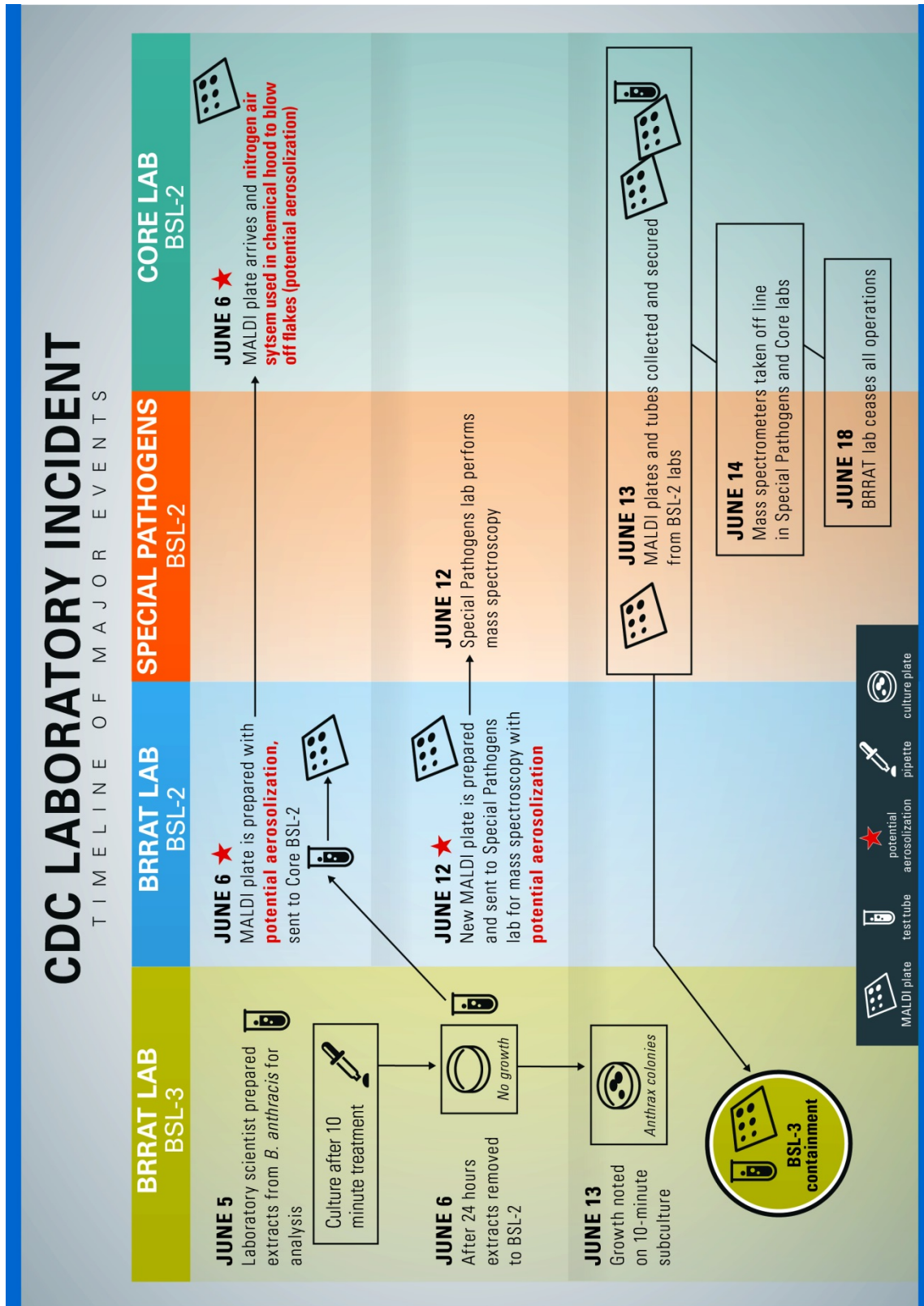
- Based on information from the laboratory studies, environmental sampling, and clinical and epidemiologic risk-assessment activities, CDC clarified potential risk groups and made new recommendations for PEP.
- To date, 29 individuals were identified as being potentially at risk because they were in affected rooms when a potential aerosolization event occurred (staff who were in a single room in the BRRAT laboratory BSL-2, on June 6 or 12; and staff who were in a single room in the BCFB laboratory, on June 6, 2014). CDC recommends that these individuals continue PEP and follow-up closely with the clinic. Any other staff who had been identified earlier as potentially exposed are determined to not be at increased risk and can discontinue or not start taking PEP. These individuals were also asked to contact the clinic for an appointment

to review their potential exposures and determine next steps. For staff on PEP determined not to have been exposed who wish still to continue PEP, the clinic will assure the availability of PEP as well as long-term health monitoring to assess adverse events.

July 7 (Monday)

- Total of 41 individuals identified as being potentially at risk. During their follow up interviews additional staff were determined to have been in the affected rooms when a potential aerosol event occurred and thus encouraged to continue PEP; these additional staff had already been recommended to start PEP as part of broader efforts to reach any potentially at risk staff.
- External laboratory obtained the same results as the CDC laboratory with regard to sterilization of vegetative cells by the method used in this incident.

Appendix C – Graphic Depiction of Major Events



Appendix D – Definitions and Terms

Animal and Plant Health Inspection Services (APHIS) – A multi-function agency within the United States Department of Agriculture (USDA) responsible for protecting and promoting U.S. agricultural health, regulating genetically engineered organisms, administering the Animal Welfare Act, and carrying out wildlife damage management activities. The Agricultural Select Agent Service (AgSAS) is part of this agency.

Biological agent – Any microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism; deterioration of food, water, equipment, supplies, or material of any kind; or deleterious alteration of the environment.

Biological Select Agent and Toxin (BSAT) – Any biological agent or biological toxin that requires registration with the Federal Select Agent Program to possess, use, and transfer. In this manual, the term, “select agents and toxins,” may also be used to mean BSAT.

Biosafety Cabinet (BSC) – The primary means of containment used for working safely with infectious microorganisms that are designed to provide personnel, environmental, and product protection.

Biosafety Level (BSL) – The risk criteria used to define the four ascending levels of containment, referred to as biosafety levels 1 through 4 based on infectivity, severity of disease, transmissibility, and the nature of the work conducted.

Decontamination – The treatment of environmental surfaces (e.g., laboratory areas) to render any infectious materials sterile and nonpathogenic.

Federal Select Agent Program (FSAP) – The joint AgSAS and DSAT program responsible for administering the select agent regulations.

Inactivation – The process of treating an infectious agent to render it non-infectious and nonpathogenic.

Responsible Official (RO) – The individual designated by an entity with the authority to ensure compliance with the Select Agent Regulations at the entity.