MINIREVIEW

Role of the Hospital-Based Microbiology Laboratory in Preparation for and Response to a Bioterrorism Event

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Many hospital-based clinical microbiology laboratories were called upon by local first responders (law enforcement, hazardous-material teams, and emergency medicine service personnel) to perform testing on powdery and miscellaneous environmental substances during the anthrax outbreak of September and October 2001. The post-September 11 anthrax outbreak was the first definitive challenge to the integrated laboratory system known as the Laboratory Response Network (LRN). The LRN strongly recommends that hospital-based laboratories restrict testing to human specimens and use only diagnostic test protocols developed in partnership with subject matter experts from the American Society for Microbiology (ASM), Centers for Disease Control and Prevention (CDC), and the Association of Public Health Laboratories. Only public health laboratories should analyze environmental and animal specimens. During the initial anthrax scare, the submission of powdery substances and other suspicious nonhuman specimens to hospital-based clinical microbiology laboratories was prompted by public panic, pressure from law enforcement to obtain an immediate answer regarding the presence or absence of anthrax spores, the unfamiliarity of first responders with the LRN, and pressure placed on laboratories to provide a public service. However, many laboratories were unfamiliar with or did not understand their role in the LRN. As a result of the anthrax experience, one of the major questions being asked by microbiologists is “what is or what should be the role of the hospital-based clinical microbiology laboratory when confronted with bioterrorism?”

DISTINGUISHING TYPES OF BIOTERRORISM EVENTS

A bioterrorism event is either overt or covert in nature. The former is self-evident and usually announced. The most notable examples of an overt event were the anthrax spore-containing letters that were sent to Senators Daschle and Leahy and to Tom Brokaw with a written note announcing that they had been exposed to Bacillus anthracis spores. The package that was sent to the National Enquirer, from which the first case of anthrax originated, is an example of a covert event. There was no indication and it was not announced that the package had been deliberately contaminated with anthrax spores. As a consequence, there was a delay in making the diagnosis of anthrax, contributing to the eventual death of the index case patient. Because an overt event involves the use of environmental substances, the hospital-based clinical microbiology laboratory should not accept these samples for testing but rather, inform the first responders that such specimens are to be tested by the local or state public health laboratories. A similar policy also applies to the testing of animal, food, and water specimens.

The hospital-based clinical microbiology laboratory should be prepared to recognize and respond to a covert event involving the collection, preservation, transport, and testing of human specimens. This type of event does not have an immediate impact because of the delay between exposure and onset of illness, i.e., incubation period. Consequently, emergency medicine physicians and other primary healthcare providers, including the clinical microbiology laboratory staff, are the sentinels or medical first responders and will most likely identify the initial cases (1). Key indicators of a possible act of bioterrorism in raising suspicion follow: (i) a disease entity that is unusual or that does not occur naturally in a given geographical area, or combinations of unusual disease entities in the same population; (ii) multiple disease entities in one patient, indicating that mixed agents have been used in the event; (iii) increased rates of morbidity and mortality relative to the num-
Large numbers of ill persons with a similar disease or syndrome
Large numbers of cases of unexplained diseases or deaths
Unusual illness in a population (e.g., unexplained respiratory infection)
Single case of disease caused by an uncommon agent (e.g., pulmonary anthrax, *Burkholderia mallei* or *Burkholderia pseudomallei*, tularemia, viral hemorrhagic fever)
Several unusual or unexplained diseases coexisting in one patient without any other explanation
Higher morbidity and mortality in association with a common disease or syndrome or failure of such patients to respond to usual therapy
Disease with an unusual geographical or seasonal distribution (e.g., tularemia in an area where it is not endemic in the summer)
Illness that is unusual (or atypical) for a given population or age group (e.g., outbreak of measles-like rash in adults)
Unusual disease presentation (e.g., pulmonary instead of cutaneous anthrax)
Similar genetic type among agents isolated from the distinct sources at different times or locations
Unusual, atypical, genetically engineered, or antiquated strain of an agent (or antibiotic resistance pattern)
Stable native disease with an unexplained increase in incidence (e.g., tularemia, plague)
Simultaneous clusters of similar illness in noncontiguous areas, domestic or foreign
Atypical disease transmission through aerosols, food, or water, which suggests deliberate sabotage
Illness limited to localized or circumscribed geographical areas
Low attack rates in personnel who work in areas with filtered air supplies or closed ventilation systems
Sentinel dead animals of multiple species
Absence of a competent natural vector in the area of the outbreak for those biological agents that are vector-borne in nature (United States Army Research Institute for Infectious Diseases [USAMRIID] Biological Warfare and Terrorism Medical Issues and Response Satellite Broadcast, 26 to 28 September 2000). Detection and recognition of a suspicious event is also dependent on a vigilant laboratory staff comprised of well-trained personnel who are capable of recognizing unusual circumstances and possess a high index of suspicion. An additional component of the recognition process includes an active surveillance and monitoring program (2).
Microbiological and epidemiological clues that may signal a potential bioterror or act of bioterrorism are summarized in Table 1 (USAMRIID Satellite Broadcast).

### ACCESSING AND INTEGRATION WITH THE LRN

The LRN is a four-tier system in which private and hospital-based laboratories, along with some local public health and military laboratories, are categorized as level A with the major responsibility being to rule out and refer suspicious isolates to the next higher level (level B, C, or D) for confirmatory testing (1, 3, 6). Laboratories designated as level B (local or state public health laboratory), level C (state health laboratories with advanced testing capacity), or level D (CDC and USAMRIID) are responsible for confirming the identification of a suspicious isolate. The level D laboratory primarily performs advanced genetic analysis, archiving, and direct analysis of specimens suspected of harboring agents requiring biosafety level 4 (BSL-4) precautions, e.g., smallpox and agents of viral hemorrhagic fever (1). Although level A laboratories have access to but are not official members of the LRN, they are regarded by public health officials as being critical to the success of the LRN. It is important that level A laboratories develop a relationship with their local and state health laboratories, many of whom provide formal training in laboratory safety, testing procedures for ruling out targeted biological agents, and clinical characteristics of diseases produced by these agents, and packaging and shipping of infectious substances. The hospital-based clinical microbiology laboratory should have a bioterrorism response plan and a standing operating procedure, which include the telephone numbers of the local and state health department laboratories and the CDC. The laboratory should have a copy of the current Targeted (Critical) Agent list developed by the CDC and its affiliated partners (Table 2). Laboratory personnel should be familiar with the bioterror categories (A, B, and C) of the different agents. Because many of these agents are uncommon, especially in the United States, or are limited to specific geographical areas, the hospital-based clinical microbiology laboratory must be familiar with the general morphological, cultural, and Gram stain characteristics of these agents and perform conventional diagnostic tests, currently listed in the LRN level A testing protocols for the bacterial agents of anthrax, brucellosis, plague, and tularemia, and rule out or refer suspicious isolates to the next higher level for confirmation of identification.

Accessing and integrating with the LRN is dependent on making improvements in the clinical and laboratory surveillance systems in addition to the training of clinicians and laboratory personnel (1, 7). To facilitate the recognition of bioterror-related microbial agents, the hospital-based clinical microbiology laboratory must attract and retain qualified personnel for conducting tests and raising awareness. Laboratory personnel should receive specialized training in the recognition of targeted agents, the use of the LRN, and the application of the level A standardized testing protocols for ruling out and referring suspicious isolates (3).
TABLE 2. Critical biological agents for bioterrorism and civilian preparedness

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>Disease, common name, or toxin</th>
<th>Biothreat level$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus anthracis$^c$</td>
<td>Anthrax</td>
<td>A</td>
</tr>
<tr>
<td>Yersinia pestis$^c$</td>
<td>Plague</td>
<td>A</td>
</tr>
<tr>
<td>Francisella tularensis$^c$</td>
<td>Tularemia</td>
<td>A</td>
</tr>
<tr>
<td>Coxiella burnet$^c$</td>
<td>Q fever</td>
<td>B</td>
</tr>
<tr>
<td>Brucella species$^c$</td>
<td>Brucellosis</td>
<td>B</td>
</tr>
<tr>
<td>Burkholderia mallei</td>
<td>Glanders</td>
<td>B</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>Salmonellosis</td>
<td>B</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>Shigellosis</td>
<td>B</td>
</tr>
<tr>
<td>Escherichia coli O157:H7</td>
<td>Hemorrhagic colitis</td>
<td>B</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>Cholera</td>
<td>B</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis$^d$</td>
<td>Tuberculosis</td>
<td>C</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variola major virus$^c$</td>
<td>Smallpox</td>
<td>A</td>
</tr>
<tr>
<td>Ebola virus$^c$</td>
<td>Hemorrhagic fever</td>
<td>A</td>
</tr>
<tr>
<td>Marburg virus$^c$</td>
<td>Hemorrhagic fever</td>
<td>A</td>
</tr>
<tr>
<td>Lassa virus$^c$</td>
<td>Lassa fever</td>
<td>A</td>
</tr>
<tr>
<td>Junin virus$^c$</td>
<td>Argentine hemorrhagic fever</td>
<td>A</td>
</tr>
<tr>
<td>VEE, VEE, EEE$^e$</td>
<td>Encephalomyelitis</td>
<td>B</td>
</tr>
<tr>
<td>Nipah virus</td>
<td>Encephalomyelitis</td>
<td>C</td>
</tr>
<tr>
<td>Hantavirus</td>
<td>Hemorrhagic fever,</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>hantavirus pulmonary syndrome</td>
<td></td>
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<tr>
<td>Tick-borne viruses</td>
<td>Hemorrhagic fever and/or</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>encephalitis</td>
<td></td>
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<tr>
<td>Yellow fever virus</td>
<td>Yellow fever</td>
<td>C</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
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<tr>
<td>Cryptosporidium parvum</td>
<td>Cryptosporidiosis</td>
<td>C</td>
</tr>
<tr>
<td><strong>Toxin sources</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium botulinum$^a$</td>
<td>Botulinum toxin</td>
<td>A</td>
</tr>
<tr>
<td>Staphylococcus aureus$^a$</td>
<td>Entertoxin B</td>
<td>B</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>Epsilon toxin B</td>
<td>B</td>
</tr>
<tr>
<td>Ricinus communis$^e$ (castor bean)</td>
<td>Ricin</td>
<td>B</td>
</tr>
<tr>
<td>Some marine dinoflagellates</td>
<td>Saxitoxin</td>
<td></td>
</tr>
</tbody>
</table>

Various species of fungi

| Trichothene mycotoxins | |

$^a$ Adapted from reference 7 with permission of the publisher.

$^b$ Biothreat level A, agents that pose the greatest threat due to their infectiousness, relative ease of transmission, or a high rate of mortality; biothreat level B, agents having a moderate ease of transmission and morbidity with a low rate of mortality; biothreat level C, emerging pathogens and potential risks for the future.

$^c$ Organisms categorized as critical agents for bioterrorism or biocrime.

$^d$ Multidrug-resistant strains.

$^e$ VEE, Venezuelan equine encephalomyelitis; VEE, western equine encephalomyelitis; EEE, eastern equine encephalomyelitis.

SAFETY

Knowledge of the BSLs is critical for the handling and management of specimens suspected of containing any of the targeted agents. Most hospital-based clinical microbiology laboratories function at BSL-2, which includes the availability of a class II biosafety cabinet (4). Once a culture or preliminary test result raises suspicion of a biothreat-related agent, all further manipulations should be performed in a biosafety cabinet. Although *Bacillus anthracis* is classified as a BSL-2 agent, the CDC and LRN recommend that all manipulations be performed in a biological safety cabinet. Therefore, all microbiology laboratory personnel should understand and be trained in the levels of biosafety (BSL-1, -2, -3, and -4).

A major reason for restricting level A laboratories to the testing of human specimens is to maintain and promote the safety of the institution, personnel, and patients. Major concerns for testing environmental samples in a hospital-based microbiology laboratory are the unknown nature of the sample. The sample could be explosive or a volatile, toxic chemical or contain a radioactive substance. Therefore, risk assessment becomes the responsibility of the clinical microbiologist, infection control personnel, hospital risk management office, and infectious disease physicians. Furthermore, a postexposure plan that describes the policy for managing laboratory or other personnel following exposure to a biothreat-related agent must be developed. Decisions must be made concerning the use of prophylactic antibiotics, administration of available vaccines, or simply instituting a fever watch policy and observing for the onset of symptoms in exposed individuals.

Of equal concern is the possibility that a specimen containing smallpox virus, a BSL-4 agent, is submitted to the virology laboratory. This virus can easily grow and amplify in most routine cell lines that are used for the cultivation of herpesviruses, e.g., varicella-zoster virus and herpes simplex virus. The technologist may neither suspect nor recognize a hazard. Failing to obtain confirmation of the more common viruses via direct fluorescent-antibody staining, the technologist may pass or manipulate the culture multiple times before terminating efforts to confirm the source of the cytopathic effects. The result is increased risk of exposure should the specimen contain smallpox virus. How to recognize and safely manage such situations is a critical challenge to the laboratory. In the event of such a circumstance, the laboratory director should call both the state health laboratory and the CDC for guidance.

COMMUNICATION

The laboratory, preferably the laboratory director, must establish and include in the laboratory bioterrorism response plan a notification policy that is enacted when a suspicious isolate cannot be ruled out and must be referred to the next higher level laboratory for confirmation of the organism’s identity. The policy must also include the point-of-contact individual within the hospital who is responsible for notifying other key personnel and the local health department officials. Event recognition within the hospital resembles an epidemiological investigation. Case definitions must be made and then followed by aggressive case analysis. In the event that a suspicious agent is detected, the first priority of the laboratory, as always, should be to notify the treating clinician of test results so that they can initiate appropriate management of patients (5, 8). To facilitate evaluation of any potential threat to the local community, the laboratory should then promptly alert the institutional infection control personnel, who in turn should notify the institutional leadership. It should be the responsibility of the infection control personnel to notify city or county public health officials, who will then notify state public health officials that an unusual illness has occurred. It is the public health authorities who will determine whether a threat to the general public health exists and who will recommend whether patient specimens or isolates should be forwarded to higher-level labora-
tories for further analysis and, if warranted, notify law enforce-
ment of followers. Under no circumstances should the laboratory
notify local, state, or federal law enforcement officials or make
the decision that a bioterrorism event has occurred. The ident-
ification of an isolate as an organism that is on the list of
potential agents of bioterrorism is not evidence that a bio-
terrorism event has occurred (8). For example, Francisella tul-
arensis, Brucella spp., and Yersinia pestis are naturally found in
certain geographical areas of the United States and are asso-
ciated with low rates of sporadic infections. Public health of-

cials and law enforcement authorities determine if an event is
creditable. The Federal Bureau of Investigation (FBI) has ul-
timate responsibility, under Presidential Decision Directive 39,
for declaring that a bioterrorism event has occurred. During
the process of determining if a threat exists to the general
public health, the clinical microbiologist should notify the
nearest LRN level B laboratory, obtain guidance concerning
the referral of the organism for confirmatory testing, and be
available to serve in an advisory capacity.

PACKAGING AND SHIPPING OF INFECTIOUS
SUBSTANCES

Specimens and agents must be packaged and shipped in
accordance with guidelines specified by the International Air
Transport Association and United Nations 6.2 packaging reg-
ulations. All specimens, including pure cultures, are classi-
ed as infectious substances and must be packaged and transported
in the same manner, whether its destination is intra- or inter-
state. Laboratory personnel must be trained and certified to
package and ship infectious substances using approved pack-
aging materials and containers (9). The laboratory director
should know which commercial carriers will accept infectious
substances, most of which will be transported to an LRN level
B laboratory. Currently, the only commercial carriers that will
accept and transport infectious substances are the United
States Postal Service and Federal Express. Although not nor-
mally a major issue for the level A clinical microbiology lab-
atory, the FBI may require that a chain-of-custody protocol be
developed for potential litigation. It is also recommended that
the laboratory retain a stock culture of any suspicious isolates
that have been referred to the next higher-level LRN labora-

tory. Isolates being saved for litigation purposes must be stored
in a secure area to prevent unauthorized personnel (e.g., en-
geineering staff, housekeeping personnel) from gaining access.

LABORATORY SECURITY

The issue and importance of laboratory security have be-
come major concerns of officials and agencies involved with the
planning of national security. In addition to maintaining the
security of the aforementioned suspicious isolates, microbiol-
ogists should give strong consideration to implementing a lab-
oratory-wide limited access policy (7). Only authorized person-
nel should have access to the laboratory. Installing an
electronic access system and requiring all members of the
laboratory staff to possess a scanning card for entry into the
laboratory can accomplish this. Unauthorized individuals
would be required to report to a central greeting area and be
screened for appropriate identification and the nature of their
business. Additional security measures include locking all stor-
age cabinets, refrigerators, incubators, and doors to sensitive
areas (e.g., tuberculosis section and virology laboratory). In
these matters, security takes precedence over convenience.

SUMMARY

In conjunction with local, state, and federal health agencies,
the hospital-based clinical microbiology laboratory is expected
to play a primary role in determining if a biocrime or bio-
terrorism event has occurred. While this role will not compromise
the routine operations of the clinical laboratory, it will enhance
their capabilities and awareness. The primary focus is to raise
the index of suspicion related to a biocrime or bioterrorism
event. In the event that the laboratory encounters a suspected
bioterrorist agent, the bioterrorism response plan is enacted.
This plan includes event recognition, access to and interaction
with the various LRN level laboratories, communication pro-

cedures, safety guidelines, training of personnel to ensure com-
petence and awareness, packaging and shipment of infectious
substances, and laboratory security.

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