Detecting Biothreat Agents: the Laboratory Response Network

This nationwide lab network coordinates surveillance and is at the ready to identify pathogens and alert authorities of suspected bioterrorist incidents

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In response to the threat of bioterrorism and following a presidential order, officials at the Centers for Disease Control and Prevention (CDC), Association of Public Health Laboratories (APHL), Federal Bureau of Investigation (FBI), and United States Army Medical Research Institute of Infectious Diseases (USAMRIID) established the Laboratory Response Network (LRN) in 1999. This national system is designed to link state and local public health laboratories with other advanced-capacity clinical, military, veterinary, agricultural, water, and food-testing laboratories, including those at the federal level. The LRN is a critical component of CDC's public health mission, enhancing U.S. readiness to detect and respond to bioterrorism incidents. In addition to biothreat agents, the LRN is expanding to include the ability to detect chemical agents.

Funding for LRN to build infrastructure, obtain state-of-the-art equipment, and train personnel is helping to rebuild the country's public health laboratory capacity, improving our ability to respond not only to bioterrorism incidents but also to other infectious diseases outbreaks. Part of these efforts include developing a well-trained laboratory workforce, including members who are certified by the American Board of Medical Microbiology (ABMM) or its equivalent, raising the capacity of LRN to respond quickly and appropriately to public health emergencies.

After officials in the Department of Health and Human Services told CDC to prepare the public health system for dealing with the bioterrorism threat, CDC developed a series of cooperative agreements with every state and several large municipalities that focus on preparedness. These agreements initially emphasized five critical areas: (i) preparedness planning and readiness assessment; (ii) surveillance and epidemiology capacity, (iii) biologic laboratory and chemical laboratory capacity, (iv) health alert network, and (v) information technology. Since the *Bacillus anthracis* bioterrorism incident of late 2001, CDC has added several critical areas, including communicating health risks, disseminating health information, education, and training.

Bioterrorism may be either covert or overt. Because a covert release of a biological agent would likely not be noticed immediately, the first signs could come days or weeks later when individuals seek medical care. Thus, the first responders will likely be astute clinicians, laboratorians, or public health workers who recognize the index case or identify the infectious agent. In the case of an announced release, traditional first responders will likely respond to actual biological agents or hoaxes. In all these cases, the incident could occur anywhere, necessitating a comprehensive public health plan for responding to bioterrorism or any infectious disease outbreak. This response will involve epidemiologic investigation, medical treatment and prophylaxis for affected persons, and other disease prevention activities. The success of these activities also depends on rapidly and accurately identifying threat agents.

The Laboratory Response Network

Operational since August 1999, the LRN builds on a longstanding, nationwide system of public health laboratories that conduct routine disease surveillance. This collaborative partnership as-

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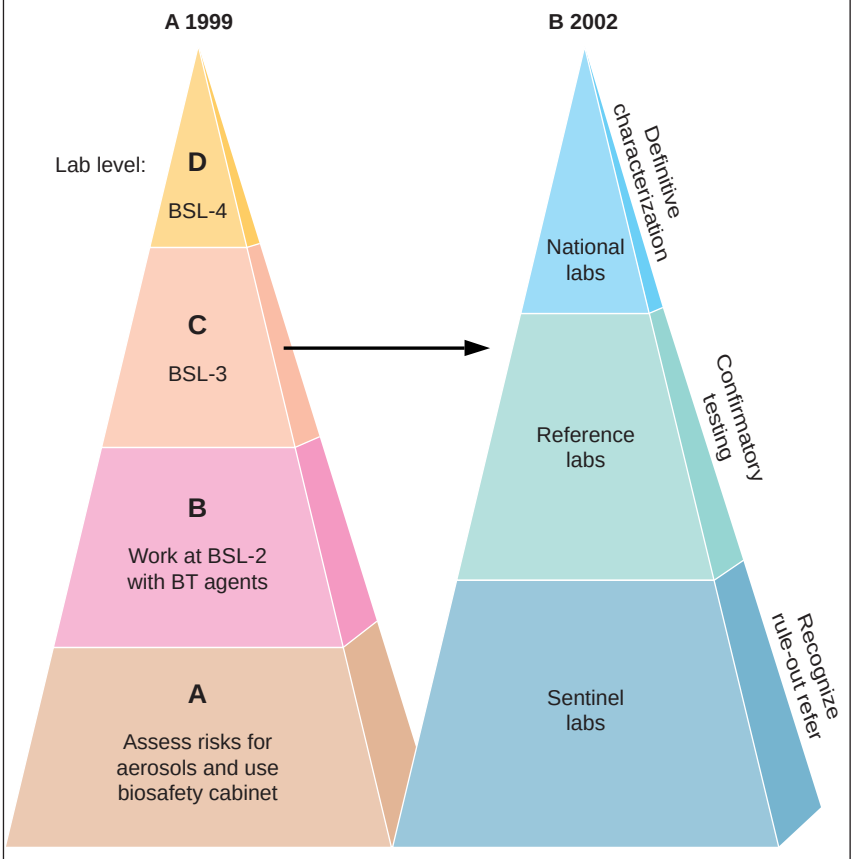
FIGURE 1



The Laboratory Response Network.

signs member laboratories as operating at either sentinel or reference levels, with those levels representing progressively stringent safety, containment, and technical proficiency capabilities (Fig. 1). A member laboratory provides its own agent-specific self-designation, meaning a particular lab may have different capacity for testing for botulinum toxin and anthrax.

Sentinel (formerly Level A) laboratories, for the most part, include hospital and other community clinical laboratories. In the aftermath of a covert bioterrorism attack, patients will seek care at widely dispersed hospitals where such local laboratories will be called upon to conduct routine testing. Typically, these laboratories would participate in the LRN by ruling out or by referring critical agents that they encounter to nearby LRN reference laboratories. To facilitate these efforts, CDC, in collaboration with ASM, developed protocols and algorithms for clinical labs to follow (Fig. 2). Algorithms for pathogens other than *B. anthracis* are available on the Internet at either www.asmusa.org or www.bt.cdc.gov.

Reference laboratories that do confirmatory testing include primarily state and local public health laboratories, with biosafety level 2 facilities where biosafety level 3 practices are observed, and public health laboratories with biosafety level 3 facilities, or certified animal facilities, which are necessary for performing the mouse toxicity assay for botulinum toxin. Some LRN reference laboratories can perform additional tests requiring biosafety level 3 containment, such as handling powders suspected of containing anthrax spores. There are 120 reference laboratories in the United States and Canada. All 50 states now have the capacity to perform tests for identifying *B. anthracis* and other potential bioterrorism agents, including *Yersinia pestis* and *Francisella tularensis,* and have ready access to specialized laboratories to confirm *Brucella* spp., *Clostridium botulinum* neurotoxin, and variola major.

Currently, two federal laboratories, at CDC and USAMRIID, are available with biosafety level 4 capacity for handling viral agents such as Ebola and variola major, for

Table 1. LRN relationships

Disciplines

Public health laboratories (federal, state, local)
Clinical microbiology laboratories
Military laboratories
Veterinary microbiology laboratories
Environmental laboratories
Food microbiology laboratories

Partnerships

Association of Public Health Laboratories
American Society for Microbiology
Federal Bureau of Investigation
Department of Defense
Food and Drug Administration
U.S. Department of Agriculture
Department of Homeland Security
Environmental Protection Agency
American Association of Veterinary Laboratory Diagnosticians

which other laboratories have insufficient safety facilities or unvaccinated staff. Federal laboratories also can perform all reference procedures. As part of their LRN responsiblities, they identify agents in specimens referred to them by reference laboratories and identify recombinant microorganisms that may not be recognizable by conventional isolation and identification methods. Federal laboratories also maintain extensive culture collections of critical agents against which the isolate(s) from a bioterrorist event may be compared.

Improvements being made in every state have led to increased capacity throughout the LRN and have fostered a maturing national asset that is a "unified network of integrated laboratories functioning through a single operational plan." Thus, it consists of laboratory first responders, or sentinel laboratories (formerly level A), and confirmatory testing laboratories at the reference level representing several disciplines (Table 1). This revised structure also reflects the system's increased technical capacity and response ability.

LRN Management, Data-Handling Capacity, Enrollment Requirements

A dedicated help desk coordinates daily operations for the LRN, providing a central clearinghouse for its 1,200 users and acting as a point of contact for labs within this nationwide network. In addition, a standing data management team addresses LRN information technology needs. For example, to support electronic reporting of proficiency testing results, this group created a database that permits LRN member laboratories to review their test results and evaluate their performances. Additionally, other databases are being developed, including one that will enable members to report test data on a secure basis and to share other critical information as well as inventory and tracking of orders.

Critical Biological Agents for Public Health Preparedness

Category A

Variola major (smallpox)
Bacillus anthracis (anthrax)
Yersinia pestis (plague)
Clostridium botulinum neurotoxins (botulism)
Francisella tularensis (tularemia)
Filoviruses (e.g., Ebola and Marburg) and arenaviruses (e.g., Lassa and Junin) (hemorrhagic fever)

Category B

Coxiella burnetti (Q-fever)
Brucella spp. (brucellosis)
Burkholderia mallei (glanders)
Alphaviruses (i.e., Venezuelan, Eastern, and Western equine encephalomyelitis viruses)
Ricin from *Ricinus communis* (ricin intoxication)
Epsilon toxin of *Clostridium perfringens*
Staphylococcal enterotoxin B
Food and waterborne agents (e.g., *Salmonella* spp., *Shigella dysenteriae, Escherichia coli* O157:H7, *Vibrio cholerae, Cryptosporidium parvum*)

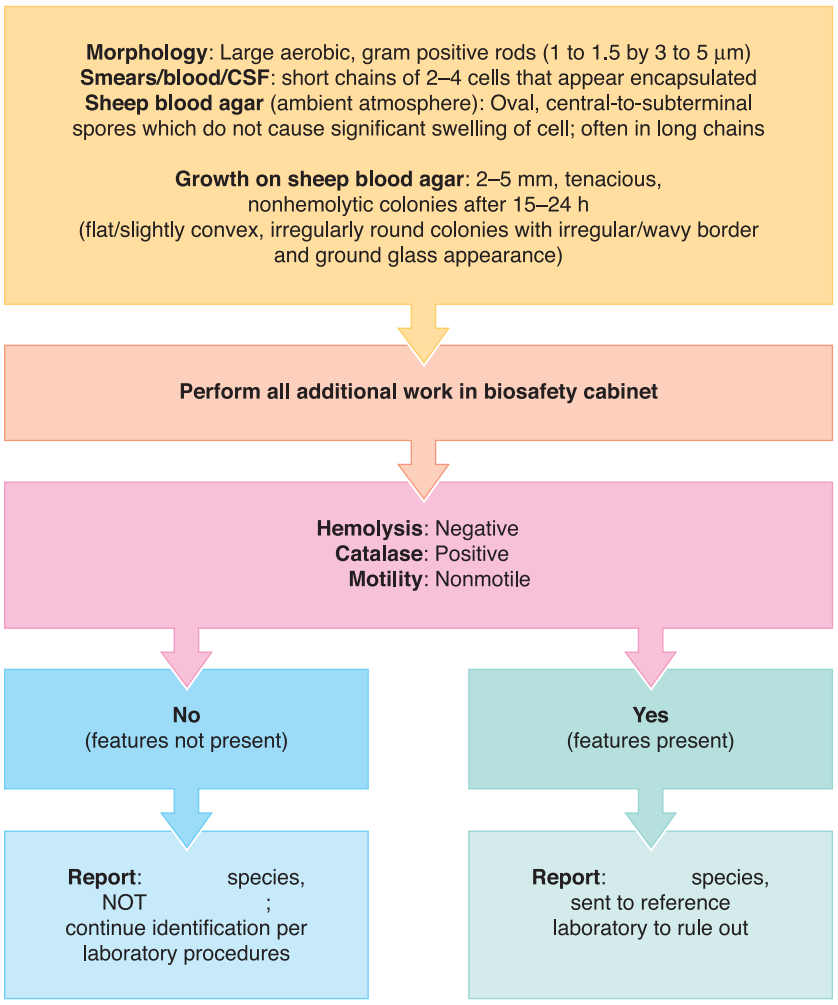
Category C

Nipah virus
Hantaviruses
Tickborne hemorrhagic fever viruses
Tickborne encephalitis viruses
Yellow fever virus
Multi-drug-resistant *Mycobacterium tuberculosis*

In assessing public health preparedness, national experts in 1999 developed criteria for ranking biological agents that threaten civilians. These criteria include the ability of such agents to: (i) cause mass casualties; (ii) be widely disseminated; (iii) be transmitted from person to person; (iv) trigger public perceptions about being attacked; and (v) require special public health preparedness steps.

Category A includes many well-recognized biowarfare agents, which are likely to cause mass casualties and require broad-based public health preparedness. Category B agents also have some potential for large-scale dissemination, but generally cause less-severe illness than those in Cate gory A. Many of these agents have been or are being weaponized. Moreover, some Category B agents could be used to contaminate food or water sources, and many of them are relatively easy to obtain. Biological agents that are not currently believed to present a high bioterrorism risk to public health, but which could emerge as future threats, were placed in Category C. Some of these agents are associated with emerging infections or are those with characteristics that could be exploited for deliberate dissemination. Officials at the National Institutes of Health also use this critical agent list as a guide for establishing bioterrorism research priorities.

FIGURE 2



LRN protocols for clinical labs.

A laboratory may be admitted into the LRN on the basis of several criteria, including: (i) its contribution to the public health needs of its state; (ii) its ability to meet safety requirements; (iii) its ability to meet federally specified physical and personnel security requirements; (iv) completion of a self-administered checklist; and (v) secure communication of test information. State public health laboratory directors must document the need for expanded capacity before additional state and local public health labs may apply for entry to the LRN.

Funding for LRN member labs comes through federal cooperative agreements, which provided $23.1 million in the first three years of this program. Following the 2001 anthrax incidents, LRN member labs received supplemental awards totaling $118 million. Besides CDC, many additional federal agencies and departments and also professional societies and public health organizations also provide resources and expertise for LRN (Table 1).

Soon after the LRN was established, member labs were called on to respond to the anthrax incidents in late 2001. On 3 October, for instance, the LRN laboratory in Jacksonville, Fla., rapidly confirmed that the pathogen isolated from the initial victim in Palm Beach County was *B. anthracis*. By late November, LRN laboratories tested more than 125,000 clinical specimens and environmental samples, requiring approximately 1 million assays; 69% were processed by public health laboratories, 25% by DoD LRN laboratories, and 6% by the CDC. Although the cases were limited to Florida, New Jersey, New York, Connecticut, and the District of Columbia, the majority of the environmental specimens tested came from states where there was no anthrax, meaning most were hoaxes or specimens provided by frightened or concerned individuals.

LRN System Specifies Protocols, Standardized Reagents

LRN labs use standard protocols and reagents to identify and confirm threat agents. CDC is mandated to develop, produce, validate, package, and ship reagents used in most of these screening and confirmatory procedures. Experts write the testing protocols that are reviewed by laboratorians. Descriptions of these reference protocols are available on a secure website managed by CDC that also contains information for ordering needed reagents and strains. Because bioterrorism involves criminal acts, specimens and cultures are handled as evidence, meaning test protocols contain information describing chain-of-custody requirements.

The Rapid Response and Advanced Technology (RRAT) laboratory at CDC is developing improved methods for identifying biothreat

Table 2. Platforms supported by the LRN

RT-PCR assays for Category A and B agents

LightCycler™, Roche Diagnostics Corporation, Indianapolis, Ind.
Smart Cycler™, Cepheid, Sunnyvale, Calif.
GeneAmp™ 5700, ABI PRISM™ 7000, and ABI PRISM™ 7700 Sequence Detection Systems, Applied Biosystems, Foster City, Calif.
iCycler iQ Real-Time PCR Detection System, Bio Rad, Hercules, Calif.

TRF assays for antigen or antibody detection for Category A and B agents

Victor2, Perkin Elmer-Wallach, Boston, Mass.

agents, focusing on real-time polymerase chain reaction (RT-PCR) assays and time-resolved fluorescence (TRF) immunoassays. The RRAT laboratory also serves the LRN as its primary source of test methods, validation data, and proficiency testing. Although LRN member laboratories decide for themselves which brand of commercially available instruments to purchase, the RRAT laboratory has adjusted the test protocols for use with a variety of such instruments (Table 2). Some identifications can be obtained within one hour using automated nucleic acid extractions and RT-PCR. The RRAT laboratory posted validated rapid nucleic acid amplification and antigen detection protocols for anthrax on the LRN website in October 2001. The LRN was also used recently to address public health emergencies associated with the smallpox vaccination campaign, as well as the SARS coronavirus and monkeypox outbreaks.

Training Is Critical for Overall Bioterrorism Readiness Effort

Having trained personnel is critical for the overall bioterrorism defense readiness effort. Initially, the LRN training focus was to build awareness among those working in sentinel laboratories to the threat of bioterrorism and to the agents of concern. For instance, a one-day course convened at eight sites brought in more than 600 individuals, and information from this course was then posted on CDC's website.

Another week-long course was designed to help personnel from state and regional public health labs learn more about identifying category A and B bacterial agents (see box, p. 435). The first offering of this course at the Georgia State Public Health Laboratory in 2000 focused on reference testing protocols for *B. anthracis*, *Y. pestis*, *F. tularensis*, and *Brucella* spp. Additional topics included the role of the RRAT Laboratory, handling hazardous evidence, and automated microbial identification systems. Additional one-week training courses for RT-PCR and TRF assays were developed and presented by early 2002. In each of these cases, participants were asked to train additional laboratorians at their home sites.

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