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PUBLIC HEALTH

Building Microbial Forensics as a Response to Bioterrorism

Bruce Budowle,[1]* Steven E. Schutzer,[2] Anja Einseln,[1] Lynda C. Kelley,[3] Anne C. Walsh,[4] Jenifer A. L. Smith,[1] Babetta L. Marrone,[5] James Robertson,[1] Joseph Campos[6]

Bioterrorists use microbes or their toxins to invoke fear, to inflict harm, and to impact economic well-being (*1*, *2*). Although microbes have been used as weapons for centuries (*3*, *4*), the anthrax letter attacks of 2001 generated great terror in the public. The attacks and subsequent public reactions revealed the need for an infrastructure with analytical tools and knowledge bases to rapidly provide investigative leads and help determine who was responsible for the crime (i.e., attribution), the source of the anthrax, and how and where the weapon was produced (Fig. 1).

There are examples of well-developed practices for handling and analyzing pathogenic agents (*5*, *6*). However, many of these assays address epidemiological concerns and do not provide sufficient information on the strain or isolate to allow law enforcement to better identify the source of the evidence sample. The continued development of additional assays for individualization of microbial strains is needed. For example, determining the microbe sent in a letter as *Bacillus anthracis* identifies the causative agent. At this point anyone who had access to *B. anthracis* is considered a potential perpetrator of the crime. But determining it was the Ames strain, an uncommon strain in nature, limits the investigation to those who had access to the specific strain and exculpates innocent scientists investigating *B. anthracis*. All of the above must be defined adequately and validated sufficiently to meet forensic needs. Furthermore, there are not many laboratories with adequate biocontainment facilities to handle forensic cases. Partner laboratories with specialty expertise will assist in investigations (see below). There is little guidance on the logistics and financial commitment required to construct a microbial forensics laboratory or to retool partner laboratories to perform microbial forensic work.

The U.S. government now has the goal of instituting a dedicated national microbial forensics system. Microbial forensics can be defined as a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime, or inadvertent microorganism/toxin release for attribution purposes. Law enforcement has had the traditional role and infrastructure for investigating crimes and is now enhancing its capabilities to confront the new challenge of biological weapon usage and bioterrorism through partnership with the scientific community. To lay a proper foundation for the field of microbial forensics, the Federal Bureau of Investigation (FBI) initiated the Scientific Working Group on Microbial Genetics and Forensics (SWGMGF) on 29 July 2002 (*7*). This working group provides an avenue for scientists from diverse disciplines within the government, academia, and the private sector to address issues collaboratively and to develop guidelines related to the operation of microbial forensics.

The FBI has hosted scientific working groups for other forensic disciplines. Perhaps the most notable is the Scientific Working Group on DNA Analysis Methods (*8*). Its success can be seen by the common use of DNA analysis in crime laboratories, the existence of standards of performance and practices, and the overwhelming acceptance of DNA analysis in the courts. Similarly, the SWGMGF aims to contribute to the infrastructure and development of tools for microbial forensics.

The members of SWGMGF, whose expertise spans multiple diverse scientific disciplines, represent a number of government agencies (*9*) and academia (*10*). Substantial input can also come from industry, and representatives from the private sector will be invited on a case-by-case basis for consultation. The cost of operations of the working group is relatively inexpensive because participants serve voluntarily.

The SWGMGF initially has focused on (i) defining quality assurance (QA) guidelines for laboratories performing microbial forensic casework analyses; (ii) establishing criteria for development and validation of methods to characterize or individualize various threat agents in ways that can be used forensically to attribute criminal acts; (iii) prioritizing efforts on those pathogens and toxins that would most likely be used in biocrimes; (iv) understanding and enhancing microbial population genetic data so that a finding can be interpreted; and (v) establishing design criteria for information databases.

Because quality practices are so important for establishing a solid foundation and maintaining credibility, the top priority was to develop a QA document for laboratories performing microbial forensic analyses. The QA guidelines document has been completed and is presented here (see *Science* Online). We address the whole laboratory infrastructure and processes encompassing the analytical typing process including organization, management, personnel education and training, facilities, security, documentation, data analysis, quality control of reagents and equipment, technical controls, validation, proficiency testing, reporting of results, auditing of the laboratory procedures, and safety.

[1]Federal Bureau of Investigation, Laboratory Division, Quantico, VA 22135, USA. [2]University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Department of Medicine, Newark, NJ 07103, USA. [3]Russell Research Center, U.S. Department of Agriculture, Athens, GA 30604, USA. [4]Wadsworth Center, New York State Department of Health, Albany, NY 12201–0509, USA. [5]Los Alamos National Laboratory, Los Alamos, NM 87545, USA. [6]Department of Laboratory Medicine, Children's National Medical Center, Washington, DC 20010, USA.
*To whom correspondence should be addressed. E-mail: bbudowle@fbi.gov.

These QA guidelines are based on the standards for human forensic DNA typing (*11*), clinical laboratories standards (*12*), and the International Standards Organization (*13*), as well as the experience of a broad range of scientists. Earlier drafts of this QA guidelines document were presented for commentary to members of several universities, public health departments, hospitals, and professional societies to obtain broad input from the scientific community. The QA guidelines must be continuously reviewed so that they can evolve on the basis of experiences and current challenges. Comments for improving these guidelines are necessary and welcomed and should be sent to the authors. We also welcome input that may facilitate implementation.

We believe these guidelines will provide a basis for uniform quality practices for laboratories performing microbial forensics work, as well as others in various fields of science. Microbial forensics draws on the expertise of many disciplines. For example, an investigation may require a microbiologist for evaluating culture morphology, a chemist for isotope analysis, a molecular biologist for genetic typing, and a forensic scientist for fingerprint analysis. Each of these scientists will need to carry out analyses under quality practice conditions appropriate to a forensic investigation. Documents such as the QA guidelines provide focus and guidance for scientists who perform analytical work. Moreover, these guidelines can serve as a template for microbiology, molecular biology, and other application-oriented laboratories. In addition, our efforts may stimulate development of new approaches and technologies.

The recommendations of the SWGMGF will be implemented in the national microbial forensics laboratory network, other partner laboratories, and, where applicable, subcontracted laboratories. The United States is developing the National Bioforensics Analysis Center (BFAC), which is part of the National Biodefense Analysis and Countermeasures Center (NBACC) and the Fort Detrick (Frederick, MD), interagency biodefense campus (*14*). The BFAC and partner laboratory network will serve as the national forensic reference center to support homeland security for the attribution of the use of biological weapons. The laboratory will be supported primarily by the Department of Homeland Security (DHS) in partnership with the FBI, and the BFAC will execute and coordinate microbial forensic casework.

To be successful, this national microbial forensic laboratory must rely on at least three major components. The first is a knowledge center composed of databases on genomics, microbiology, forensics methods, associated materials, and related evidence assays (including traditional forensic analyses such as fingerprints), bioinformatics, and standardized tools. The second component is the maintenance of strong partnerships between existing government, academic, and private-sector assets. These will include Plum Island, Department of Defense, Department of Energy, Department of Health and Human Services, National Science Foundation, National Laboratories, specialty technology laboratories, and other centers of excellence. No single laboratory or institution can address all microbial forensic needs. Although the FBI has at times reached outside its own laboratory for scientists to provide assistance in casework, analysis of materials from the anthrax letter attacks may be the first time that so many outside scientists with diverse expertise were employed. This may well be standard practice in future cases. The third component is the SWGMGF. The SWGMGF's first contribution to the BFAC and bioforensic network is these QA guidelines. All of these components will form a partnership network with the capability of efficiently investigating potential bioterrorist activity (Fig. 2).

In conclusion, scientists can play a substantial role in thwarting the use of bioweapons by developing tools to detect and to determine the source of the pathogen and to identify those who use such biological agents to create terror or to commit crime. By developing a robust microbial forensics field, security can be enhanced beyond physical locks and barriers.

References and Notes

1. National Research Council and Committee on Science and Technology for Countering Terrorism, *Making the Nation Safer: The Role of Science and Technology in Countering Terrorism* (National Academy Press, Washington, DC, 2002).
2. J. Lederberg, *Science* **288**, 287 (2000).
3. R. J. Hawley, E. M. Eitzen Jr., *Annu. Rev. Microbiol.* **55**, 235 (2001).
4. W. S. Carus, "Bioterrorism and biocrimes: The illicit use of biological agents since 1900," Working Paper, National Defense University; available at http://www.ndu.edu/centercounter/Full_Doc.pdf.
5. U.S. Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories* (Government Printing Office, Washington, DC, ed. 4, April 1999).
6. D. O. Fleming, D. L. Hunt, *Biological Safety Principles and Practices* (ASM Press, Washington, DC, ed. 3, 2000).
7. For further information see www.promega.com/profiles/601/ProfilesInDNA_601_07.pdf
8. B. Budowle, *Crime Lab. Dig.* **22**, 21 (1995).
9. Central Intelligence Agency, Centers for Disease Control and Prevention, Department of Homeland Security, Department of Justice, Department of Defense, Federal Bureau of Investigation, Food and Drug Administration, Los Alamos National Laboratory, Lawrence Livermore National Laboratory, National Academy of Science, National Institutes of Health, National Science Foundation, New York State Department of Health, Office of Science and Technology Policy, and U.S. Department of Agriculture.
10. Children's National Medical Center, North Carolina State University, Northern Arizona University, University of Cincinnati, University of Louisville, University of Medicine and Dentistry of New Jersey–New Jersey Medical School, and The Institute for Genome Research.
11. FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories* (Government Printing Office, Washington, DC, 1998).
12. Clinical Laboratory Improvement Amendments 1988 (CLIA '88). Code of Federal Regulations (C.F.R.), 57 C.F.R. 7139, 883 (2001).
13. International Standards Organization (ISO)/International Electrotechnical Commission (IEC), "General requirements for the competence of testing and calibration laboratories" (ISO/IEC 17025, American National Standards Institute, New York, 1999), 26 pp.
14. B. Budowle, J. Burans, M. R. Wilson, R. Chakraborty, R., In: *Microbial Forensics*, S. Shutzer, R. Breeze, and B. Budowle, Eds. (Academic Press, San Diego, in press).
15. This is publication number 03-12 of the Laboratory Division of the Federal Bureau of Investigation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/VOL/ISSUE/PAGE/DC1

Fig.1. Techniques that can be used to analyze evidence and its components.

Fig. 2. Partnership network. Microbial evidence, either from real events or from hoaxes, may enter the bioforensic laboratory network by different routes. If an event is immediately recognized as an act of bioterrorism, any evidence will be sent directly by first responders, the intelligence community (IC), or the Department

of Defense (DoD) to the national bioforensic laboratory. Alternatively, an event may be thought to be naturally occurring and therefore evidence will be sent to the public health sector, i.e., the Laboratory Response Network (LRN) of the Centers for Disease Control and Prevention (CDC). Once the evidence is deemed to be from an act of bioterrorism, the materials will be sent by the LRN to the national bioforensic laboratory for attribution analysis. That laboratory will carry out a suite of applicable assays, as well as use the partnership network to enhance attribution characterization capabilities.