

Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters

It is not possible to reach a definitive conclusion about the origins of the *Bacillus anthracis* used in 2001 anthrax mailings based on the available scientific evidence alone, this National Research Council committee finds. Scientific analysis played a central role in the FBI's investigation of the anthrax mail attacks. To help investigators narrow their search for the source of the attack material, researchers characterized its physical, chemical, and biological properties. In this report the committee reviews the scientific approaches used during the investigation, and evaluates whether the FBI reached appropriate scientific conclusions from the use of these techniques.

In the fall of 2001, letters containing anthrax spores were mailed to news media and congressional offices, killing five people and sickening 17 others. In response to the attack, the Federal Bureau of Investigations (FBI) launched an extensive investigation that spanned six continents, involved over 10,000 witness interviews, and resulted in the issuance of some 5,750 grand jury subpoenas. During the investigation, the FBI worked with other federal agencies to coordinate and conduct scientific analyses of the anthrax spore powders, environmental samples, clinical samples, and samples collected from laboratories that may have been the source of the letter-associated spores. In addition, several government, university, and commercial laboratories assisted in conducting the scientific analyses that were a central aspect of the investigation.

Although it is not unusual to use science to investigate evidence in a criminal case, tracing the source of the anthrax spores used in the mailings would require the development of new scientific techniques. In addition to informing FBI investigators about possible leads, much of the science used in the investigation formed the basis of a rapidly developing but still nascent

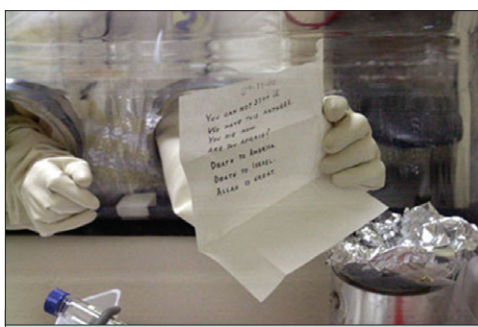


Figure 1. Letter containing anthrax mailed to Senator Patrick Leahy on October 9, 2001.

Source: FBI

scientific field called microbial forensics, which serves to determine the genetic identity of a microbial agent used for nefarious purposes.

In 2008, seven years into the investigation, the FBI asked the National Research Council to conduct an independent review of the scientific approaches used during the investigation of the anthrax mailings. A

committee of experts evaluated the scientific foundation for the experiments conducted by, and on behalf of the FBI, to determine whether they met appropriate standards for scientific reliability and for use in forensic validation, and to determine whether the FBI reached appropriate scientific conclusions from its use of these techniques. The committee did not undertake an assessment of the probative value of the scientific evidence and did not offer any view on the guilt or innocence of any individual(s) in connection with the 2001 mailings.

Looking for Leads

Investigators analyzed the physical and chemical characteristics of the powders recovered, performing experiments to study the size and granularity of the particles, to assess the chemical content and age of the spores, and to identify any other chemical signatures that

Box 1. What is anthrax?

Anthrax is a disease caused by the bacterium *Bacillus anthracis*. It is generally a disease of herbivores such as cows and sheep, which acquire the infection by grazing on contaminated soils. Highly resistant to environmental degradation, *B. anthracis* spores can survive for long periods of time in soil, their natural reservoir. The disease occurs worldwide and there are occasional outbreaks in livestock in the United States.

Humans can acquire *B. anthracis* infection via the skin, gastrointestinal tract, or by inhalation. Most commonly, this occurs by eating the meat or handling the hides of infected animals.

B. anthracis infections caused by the inhalation of *anthracis* spores are the most lethal. The high mortality rate, and the fact that with appropriate preparation, prodigious quantities of the spores can be produced and converted to an easily inhaled powder, mean that *B. anthracis* spores are effective agents of biological warfare and bioterrorism.

might provide clues related to the source or production processes used. The committee found that these analyses were appropriate and were carried out properly, and agreed with the FBI and Department of Justice that these analyses proved to have limited forensic value in identifying the source of the anthrax.

Researchers also carried out tests to determine if the spores had been weaponized by coating them with a chemical such as silicon to help the spores disperse into the air once the envelopes were opened. Although silicon was present in the letter powders, the committee found no evidence of the intentional addition of silicon-based dispersants.

Tracing the Anthrax

There are many different strains of *B. anthracis*, each with distinct genetic features, which have evolved over time. Before the anthrax mailings took place, scientists had developed molecular methods



Figure 2. Anthrax powder recovered from the *New York Post* letter. Source: FBI

to search the genes of anthrax spores for markers to differentiate the various strains. Using these tests, researchers identified the dominant organism in the letters as the Ames strain of anthrax. Because the Ames strain had been widely distributed among laboratories around the world for research and countermeasure development (see box 2), this finding suggested that the attack material had come, either directly or indirectly, from one of the laboratories that possessed the Ames strain. Further analysis of the genome sequence of the letter-associated anthrax spores determined that it was unlikely that this strain had been genetically engineered—a concern for investigators who feared the strain may have been altered to enhance antibiotic resistance or to increase virulence.

Searching for the Source

Having determined that the anthrax used in the letters was the Ames strain, the FBI worked to establish a repository of Ames strain samples held in laboratories around the world. In all, 20 laboratories provided a total of 1070 samples of Ames strain anthrax stocks to the FBI.

To identify the source of the letter materials, researchers searched for a link between the material recovered from the letters and samples in the repository. Microbiologists at USAMRIID had observed that some spores from the attack material grew to produce bacterial colonies with distinctive appearances, different from typical Ames strain anthrax colonies. These variants were suspected to be the result of genetic mutations that had spontaneously arisen in the attack spore population.

Researchers determined the nature of the mutations associated with some of the unusual colonies in the attack material, and then designed tests to search the FBI's repository of anthrax stocks for samples that contained these same mutations. The tests showed that 8 samples from

Box 2. What is the Ames strain?

The Ames strain of *B. anthracis* was first isolated from a dead cow in Texas in 1981. Texas A & M University shipped this new isolate to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) in Fort Detrick, Maryland. Because the box used for the shipment bore a previously-used Ames, Iowa, address label, the strain became known as the “Ames” strain. Subsequently, the Ames strain was shared with laboratories and research institutions throughout the United States and the world.

two laboratories contained the same set of four mutations that had been selected from the attack material as the basis for subsequent tests. The FBI, through its other investigatory efforts, stated that all of these samples derived from a flask labeled RMR-1029, found in a laboratory at USAMRIID.

A bacterial colony is a visible cluster of bacteria growing on a solid medium such as an agar plate. All the cells in one colony are descendents of a single cell, and are therefore genetically identical.

Evaluating the Science

The committee agreed that genetic analysis of the FBI's repository was consistent with the finding that the spores in the attack letters came from flask RMR-1029; but the analyses did not definitively demonstrate such a relationship. Overall the committee found that the scientific link between the letter material and flask RMR-1029 is not as conclusive as stated in the Department of Justice's Investigative Summary, for the following reasons:

- The FBI's repository was not optimal. For example, instructions provided to laboratories for preparing samples for the FBI's repository were not precise enough to ensure that all the laboratories would follow the same procedure. The lack of consistently prepared samples limits the strength of comparisons of the repository samples and the letter material. Furthermore, the instructions were not sufficient to ensure that samples of atypical Ames colonies, such as those found in the anthrax letters, would have been submitted to the repository by recipients of the FBI's instructions.
- It is possible that some of the mutations identified in the attack materials and in flask RMR-1029 could have arisen independently, by parallel evolution. This particular type of mutation is known to arise frequently and be enriched during large-scale *B. anthracis* growth procedures and spore preparation. This possible explanation for the genetic similarity between the spores in the letters and in RMR-1029 was not rigorously explored during the course of the investigation.
- Flask RMR-1029, identified by the U.S. Department of Justice as the "parent material" for the spores in the attack letters, was not the



Figure 3. Bacterial colonies formed by growth of *Bacillus anthracis* cells on blood agar. The colony on the top displays a "bull's eye," typical of *anthracis* colonies. The bottom colony displays an appearance more typical of the Ames strain.

Source: U.S. Army Medical Research Institution of Infectious Diseases

immediate source of the spores used in the letters. As noted by the FBI, one or more derivative growth steps would have been required to produce the anthrax in the attack letters. Furthermore, the contents of the New York and Washington letters had different physical properties.

The committee found the FBI's scientific data provided leads as to the origin of the anthrax spores in the letters, but these data alone did not rule out other possible sources. The committee was not charged with reviewing, nor was it given access to, the findings from the criminal investigation component of this case; therefore, the committee could not fully assess the potential value of additional scientific investigation with respect to establishing the source of the anthrax spores.

Late in its study, in discussions with FBI and Department of Justice leadership, the committee was made aware of additional information that would require review of classified material. Due to the timing of this revelation and the desire that all materials considered in this study be publicly available, the committee did not review these materials. A separate review of the classified materials offered by the FBI and Department of Justice should be conducted.

Looking to the Future

While much of the committee's effort was focused on a review of the science performed in support of the investigation of the 2001 anthrax

mailings, an equally important goal was to help ensure that future scientific investigations of biological attacks be conducted in the most relevant, rigorous, and thoughtful manner possible.

In the future, it will be important to ensure more timely results, more efficient environmental analysis, access to globally representative strain collections, and a robust capacity for characterizing less well-studied or easily cultivated biological agents. Officials should also ensure that the general public, policymakers, and the scientific community understand the goals of forensic science and the limitations of its use in the investigation of a biological attack, and have realistic expectations about the conclusions that can be drawn from such investigations. Through its analysis, the committee has learned several lessons from the Anthrax investigation that may be helpful in future events:

- The scientific investigation of any future biological attack would greatly benefit from a robust process of independent oversight and ongoing review. To accomplish this, the government could maintain a standing body of scientific

experts with proper security clearance who are fully briefed on matters of importance for preparedness and response against a biological attack. When an investigation is launched, members of this group could help guide the scientific investigation.

- It will be important in the future that relevant agencies review and periodically update the appropriate protocols and experimental designs to use best strategies for collecting, preserving, and documenting evidence, exploiting samples for scientific information, and meeting subsequent legal challenges.
- As was done in the anthrax investigation, at the outset of any future investigation, the responsible agencies will need to develop a scientific plan and decision tree considering the breadth of available physical and chemical analytical methods. The plan will also need to allow for the modification of current methods and for the development and validation of new methods.

Read or purchase this report and locate information on related reports at
<http://dels.nas.edu/bls>

Committee on Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 *Bacillus Anthracis* Mailings: **Alice P. Gast**, (*Chair*), Lehigh University; **David A. Relman**, (*Vice Chair*), Stanford University School of Medicine, and Veterans Affairs Palo Alto Health Care System, CA; **Arturo Casadevall**, Albert Einstein College of Medicine; **Nancy D. Connell**, University of Medicine and Dentistry of New Jersey; **Thomas V. Inglesby**, Center for Biosecurity, University of Pittsburgh Medical Center, University of Pittsburgh Schools of Medicine and Public Health; **Murray V. Johnston**, University of Delaware; **Karen Kafadar**, Indiana University; **Richard E. Lenski**, Michigan State University; **Alice C. Mignerey**, University of Maryland; **David L. Popham**, Virginia Polytechnic Institute and State University; **Jed S. Rakoff**, Southern District of New York; **Robert C. Shaler**, Pennsylvania State University; **Elizabeth A. Thompson**, University of Washington.; **Kasthuri Venkateswaran**, California Institute of Technology Jet Propulsion Laboratory; **David R. Walt**, Tufts University and Howard Hughes Medical Institute; **Anne-Marie Mazza** (*Study Director*), **Frances E. Sharples** (*Study Co-Director*, until 1/11), **Erica McGowan** (*Program Officer*, Board on Chemical Sciences and Technology, until 4/10), **Steven Kendall** (*Senior Program Associate*, Committee on Science, Technology, and Law), **Amanda Cline**, (*Senior Program Assistant*, Board on Life Sciences, until 5/10), National Research Council.

The National Academies appointed the above committee of experts to address the specific task requested by the Federal Bureau of Investigations. The members volunteered their time for this activity; their report is peer-reviewed and the final product signed off by both the committee members and the National Academies. This report brief was prepared by the National Research Council based on the committee's report.



For more information, contact the Board on Life Sciences at (202) 334-2187 or visit <http://dels.nas.edu/bls>. Copies of *Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters* are available from the National Academies Press, 500 Fifth Street, NW, Washington, D.C. 20001; (800) 624-6242; www.nap.edu.

*Permission granted to reproduce this brief in its entirety with no additions or alterations.
Permission for images/figures must be obtained from their original source.*