

## Science Needs for Microbial Forensics: Developing Initial International Research Priorities

**MICROBIAL FORENSICS HAS BEEN DEFINED** as “a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime, or inadvertent microorganism or toxin release for attribution purposes”—where attribution means determining “who did it.” This emerging discipline seeks to offer investigators the tools and techniques to support efforts for attribution in the event of a release of a biological agent. But microbial forensics is still in the early stages of development and faces substantial scientific challenges to provide a robust suite of technologies for identifying the source of a biological threat agent and attributing a biothreat act to a particular person or group. This report, based partly on a workshop held in Zagreb, Croatia, identifies scientific needs that must be addressed to improve the capabilities of microbial forensics to investigate infectious disease outbreaks and provide evidence of sufficient quality to support legal proceedings and the development of government policies.

**Through TV shows such as *CSI*,** many of us have become familiar with forensics: the use of science to gather evidence that helps solve crimes, exonerate the innocent, or identify missing persons. Often that evidence consists of human DNA or fingerprints. But when the weapons used are pathogenic microbes, a different type of forensic science is needed. Microbial forensics focuses on the biological signatures of microbes—life forms too tiny to see—to investigate biological crimes. The challenges for microbial forensics would be difficult enough within the law enforcement context of a single country, but if a suspected deliberate outbreak spanned national boundaries, the challenges would be even greater.

Because microbial forensics is an emerging discipline that is still developing, the Zagreb workshop aimed to increase awareness of microbial forensics among the members of the larger international scientific communities and elucidate the major issues that need to be addressed for the global development of the science of microbial forensics. The report’s authoring committee also gave particular attention to those areas, such as increased scientific knowledge about microbial communities and common standards and protocols for analysis, which would benefit from international cooperation and collaboration.

### **UNDERSTANDING THE MICROBIAL WORLD**

Microbes are everywhere: there are ten-million-fold more microorganisms on our small planet than there are stars in the visible universe. Yet little is known

about most of the microbial world—and much of what we do know comes from the very few microorganisms that can be cultured in laboratories.

In recent years, the new techniques of metagenomics have allowed scientists to directly assay microbial gene sequences from environmental and clinical samples, circumventing the inability to culture most microbes. But these techniques are still being developed, and meanwhile, there remains much to learn. The dearth of information about the vast majority of microbes represents a major scientific knowledge gap. For example, in the event of a biothreat scientists need baseline information on the natural abundance and distribution of the pathogen to help figure out if the presence of pathogen is natural or the result of a deliberate or inadvertent release.

### **CLINICAL AND PUBLIC HEALTH CONSIDERATIONS**

The technologies for microbial forensics could aid not only law enforcement and policy makers, but also public health workers in trying to identify the existence and source of infectious disease outbreaks. As was the case in the 2001 anthrax attacks, the public health system will likely be the first to encounter a biological attack—and the first line of defense against it.

Although the use of molecular techniques to identify pathogen species and strains has become fundamental to microbial forensics over the past 10 years, it is only now being adopted in clinical medicine. Some hospitals are, for example, now using small

benchtop sequencers to determine the identity and characteristics, such as presence of antibiotic resistance genes, in organisms infecting patients. Expanding capabilities for detecting and responding to the whole spectrum of natural, intentional, and accidental outbreaks of disease—not just the rare event of a serious biological attack—will ensure that the relevant tools and procedures are used frequently and available when they are needed in the event of a bioterrorist attack.

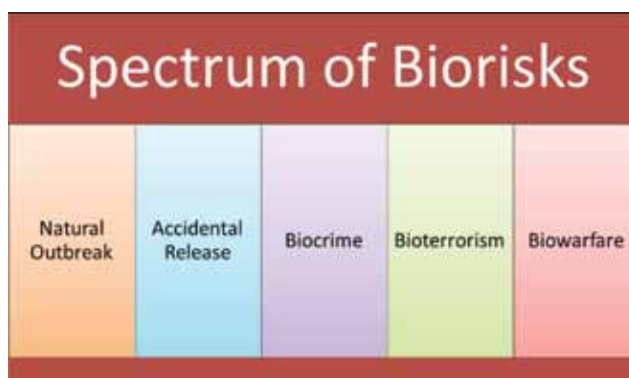
### **DEVELOPMENT OF HIGH-CONFIDENCE METHODOLOGIES TO DISTINGUISH AMONG NATURAL, ACCIDENTAL, AND DELIBERATE OUTBREAKS OF INFECTIOUS DISEASE**

Although there are criteria for considering whether a disease outbreak is unusual, determining whether such an event is due to natural, accidental, or deliberate causes, and collecting the information to work through these criteria at the time of an outbreak event is likely to be time-consuming and slow. A biological event demands rapid and effective responses to minimize loss of life, spread of illness, and to thwart criminal or terrorist activity and prevent follow-on attacks. Time lines for making this crucial determination are currently unacceptable from both public health and law enforcement standpoints, which require development and management of appropriate and rapid responses, recovery measures, and resolution.

### **METHODS AND TECHNOLOGIES**

Methods of great value in microbial forensics include both molecular genetic and non-genetic technologies. PCR, sequencing of 16S ribosomal RNA, and whole genome sequencing are molecular genetic technologies used to characterize microbial agents. Other techniques from the physical sciences, such as mass spectrometry and electron beam-based methods, can be used to analyze the physical properties of microbial forensic evidence, such as the presence of additives to enhance dispersability, or physical signatures from the locale where the material originated.

The technology and validation procedures used during the investigation of the October 2001 anthrax letters case took eight years to fully develop. In the event of a future outbreak, a timeline that long will not be acceptable. Therefore, the work of discovering more information on pathogens and pathogenicity and developing new analytical methods for microbial forensics requires a much higher priority now. Development of more advanced, faster, and cheaper assay and sequencing technologies that can be standardized and made more accessible worldwide to benefit both microbial forensics and public health is also necessary.



**Figure 1.** Both biocrimes and bioterrorism exist on a continuum of risk associated with biological agents. The other end of the spectrum deals with natural outbreaks or accidental releases of infectious disease agents. In all cases on the spectrum, public health protection requires that the microorganism first be identified and its source located to stop further cases of exposure. To this degree, medicine, public health, and law enforcement initially have common aims and methods. Microbial forensics, however, has requirements and needs that, in many ways, go beyond those of medicine and public health. Though often applicable to medicine and public health, the methods used in microbial forensics delve deeper into identification of organisms, require standardization and validation, and must meet legal standards for evidence. At the same time, the commonalities with public health provide opportunities to leverage methods and information across fields.

### **SAMPLING**

Special techniques and methods are needed to gather and preserve microbial forensic evidence—and to protect the health and safety of the first responders who gather this evidence at the crime scene. Developing and validating sample collection, preservation, handling, storage, packaging and transportation methods and procedures requires a much higher priority. Existing processes need to be standardized, compiled, and shared worldwide, while new, more efficient ones should be sought.

### **VALIDATION AND STANDARDS**

All components of a microbial forensics investigation need to be validated—from methods for collection and sampling, preservation and handling, to identification of the agent. Establishing criteria and requirements for validation, and compiling a list of all validated protocols in use (e.g., for sampling, DNA extraction and isolation, and sequencing) would help ensure the quality of microbial forensic evidence.

### **BIOINFORMATICS AND DATA**

As new sequencing methods generate huge amounts of data, needs are growing for novel ways to handle and analyze this information. Advances in bioinformatics—an interdisciplinary field that develops and

improves on methods for storing, retrieving, organizing, and analyzing biological data—are needed to help meet these needs.

Refinement of bioinformatics and statistical methods for evaluating evidence in microbial forensics is needed, including new algorithms that scale to very large data sets. Bioinformatics also needs to be made understandable and user-friendly to laboratory users, first responders, the public, and policy makers. Setting out clear requirements for the field could help attract industry to develop the hardware and software for bioinformatics, as well as the data storage mechanisms that will be necessary. A dedicated facility to test and evaluate software for bioinformatics could potentially serve the entire microbial forensics and epidemiological communities.

### **DATA SHARING**

Data sharing—from biological information on gene sequences to software, protocols, and standard methods for microbial forensics procedures—is critical. Sharing such data has the potential to promote international collaboration and cooperation among scientists, and, more importantly, inspire innovation. An international body that has the respect of the international political and scientific communities should begin discussions about how to share microbial forensic data soon.

### **REFERENCE COLLECTIONS**

Establishing a comprehensive archive of reference materials—including organisms, nucleic acids, and sequence information—could facilitate the development of standardized nomenclature and techniques and provide references for genomic comparisons. Such a facility should take advantage of existing models, such as the American Type Culture Collection and the World Data Centre for Microorganisms. A model system for a consortium of reference collections and data storage centers could be created and later scaled up to become more inclusive.

### **TRAINING AND EDUCATION**

Microbial forensics is still a relatively new discipline. Training is needed for a number of purposes, including:

- Increasing the availability of trained microbial forensics practitioners.
- Increasing the awareness and preparedness of first responders, which is essential for both safety purposes (to prevent accidental exposures of responder personnel to hazardous pathogens) and for law enforcement needs to ensure that evidence samples are not compromised and crime scenes are not contaminated.

- Improving the understanding of policy makers and the public about what microbial forensics is and what it can and cannot accomplish.

### **MICROBIAL FORENSICS NEEDS**

In its efforts to identify what science is required to develop microbial forensics further, the committee used a generous definition of “science,” including research to improve fundamental scientific understanding of microbes, specialized research intended for particular applications in public health, law enforcement, or elsewhere, and an array of technologies and methods that support both basic and applied research. The committee also identified a variety of procedural and policy needs, such as common understandings and protocols for collecting and managing samples within and among nations. The list of needs identified by the committee is long, but the successful development of microbial forensics will require addressing all of them.

### **CHALLENGING TASKS AND/OR LONG LEAD TIMES**

Some tasks are particularly challenging and/or require a long lead time to achieve the desired results. Such efforts will require the involvement of governments to provide research resources to over many years, and should be given priority by participating institutions.

- An international collaboration engaging the worldwide scientific community in a systematic effort to identify, monitor, and characterize a far higher proportion of global microbial species to increase knowledge about endemism and background. The effort should begin with known pathogens and then expand to their close relatives as well as emerging pathogens.
- Development of high confidence methods to distinguish among natural, accidental, and deliberate outbreaks of infectious disease.
- Increased emphasis on development and validation of processes (sample collection, preservation, handling, storage, packaging, and transportation) and analytical methods for microbial forensics, including establishing standards for most components.
- Discussions under the auspices of an international body that has the respect of the international political and scientific communities about how to share microbial forensic data, and for developing and presenting cogent arguments that can be persuasive to political leaders and scientists worldwide.
- An international effort to design and establish more systematic and comprehensive reference collections and databases for pathogens and other microorganisms. This effort could take advantage of existing models, such as the World Data Centre

for Microorganisms and the American Type Culture Collection. A model system for a consortium of reference collections and data storage centers could be created and later scaled up to become more inclusive.

### ONGOING EFFORTS ON WHICH TO BUILD

Some needs could take advantage of ongoing efforts to advance the development of microbial forensics, but will require deliberate communication efforts and in some cases funding to ensure that microbial forensics applications are actually included and implemented.

- Increased emphasis on research to determine mechanisms of pathogenicity, including virulence factors and host immune responses.
- Priority research to realize the promise of metagenomics and its application to microbial forensics and the development of the forensic value of the other “omics”: proteomics, metabolomics, transcriptomics, glycomics, immunogenomics, etc.
- Greatly improved global disease monitoring and surveillance in humans, animals, and plants to facilitate rapid response and better disease control.
- Improved worldwide access to molecular diagnostics (PCR, WGS, etc.), including refinement and distribution of bench-top NGS instruments that are fast and affordable and have simple workflow procedures.

- High priority continued research and development to improve physical science applications to microbial forensics.
- Refinement of bioinformatics and statistical methods for evaluating evidence in microbial forensics, including new algorithms that scale to very large or complex data sets.

### SHORTER LEAD TIMES OR INDUSTRY INCENTIVES

Some tasks have either a relatively short lead time to substantial progress, or significant markets that will provide incentives for industry.

- Development of more advanced, faster, and cheaper assay and sequencing technologies that can be standardized and made more accessible to benefit both microbial forensics and public health.
- A compilation of all protocols in use (e.g., for sampling, DNA extraction and isolation, sequencing, etc.) and whether and how they have been validated.
- Expansion of technically-based training to “professionalize” microbial forensics and increase the number of qualified practitioners worldwide by engaging international professional organizations or other entities that have experience providing training in related fields.

Locate additional information, including related reports, at <http://dels.nas.edu/bls>

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**Committee on Science Needs for Microbial Forensics: Developing an Initial International Roadmap:** John D. Clements (*Chair*), Tulane University; Munirul Alam, International Center for Diarrhoeal Diseases Research, Bangladesh; Bruce Budowle, Institute of Applied Genetics, University of North Texas Health Science Center, Fort Worth; Jongsik Chun, Seoul National University; Nancy D. Connell, Rutgers New Jersey Medical School, Rutgers University; Rita R. Colwell, University of Maryland, College Park; Paul Keim, Northern Arizona University, Flagstaff; Juncai Ma, WFCC-MIRCEN World Data Center of Microorganisms (WDCM), Institute of Microbiology, Chinese Academy of Sciences, Beijing; Alekma Markotić, University Hospital for Infectious Diseases, Zagreb; Geoffrey Smith, Imperial College London; Frances E. Sharples (*Study Director and Director*), Jo L. Husbands (*Scholar/Senior Project Director*), Carl Anderson (*Program Associate*), Board on Life Sciences, National Research Council; Benjamin Rusek (*Program Officer*), Committee on International Security and Arms Control); Kristin White (*Consultant Writer*).

The National Academies appointed the above committee of experts to address the specific task requested by the U.S. Navy, U.S. Department of State, and the National Academy of Sciences. 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 *Science Needs for Microbial Forensics: Developing Initial International Research Priorities* are available from the National Academies Press, 500 Fifth Street, NW, Washington, D.C. 20001; (800) 624-6242; [www.nap.edu](http://www.nap.edu).

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