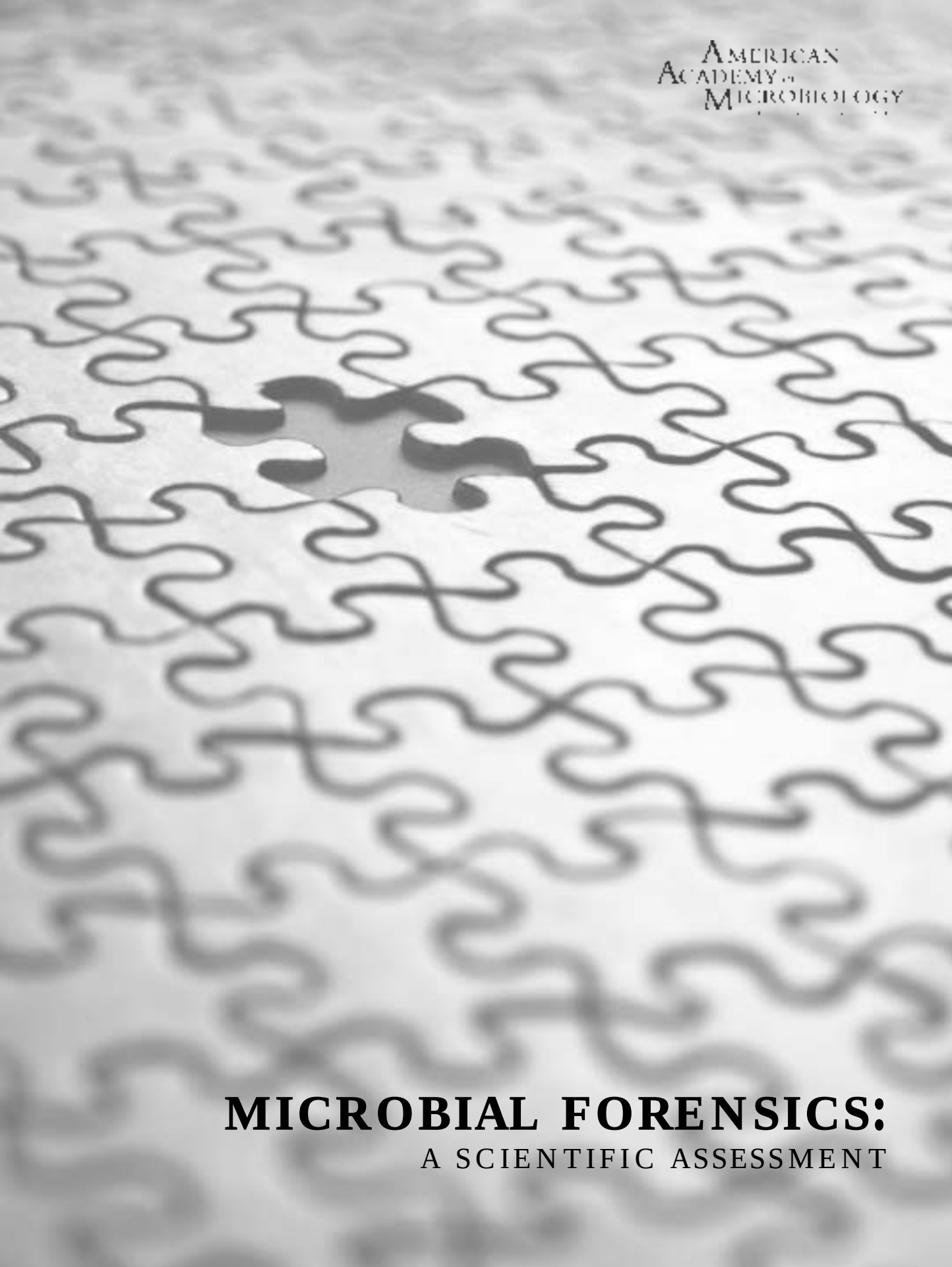

American Academy of Microbiology

MICROBIAL FORENSICS:

A SCIENTIFIC ASSESSMENT

This report is based on a colloquium sponsored by the American Academy of Microbiology held June 7-9, 2002, in Burlington, Vermont.

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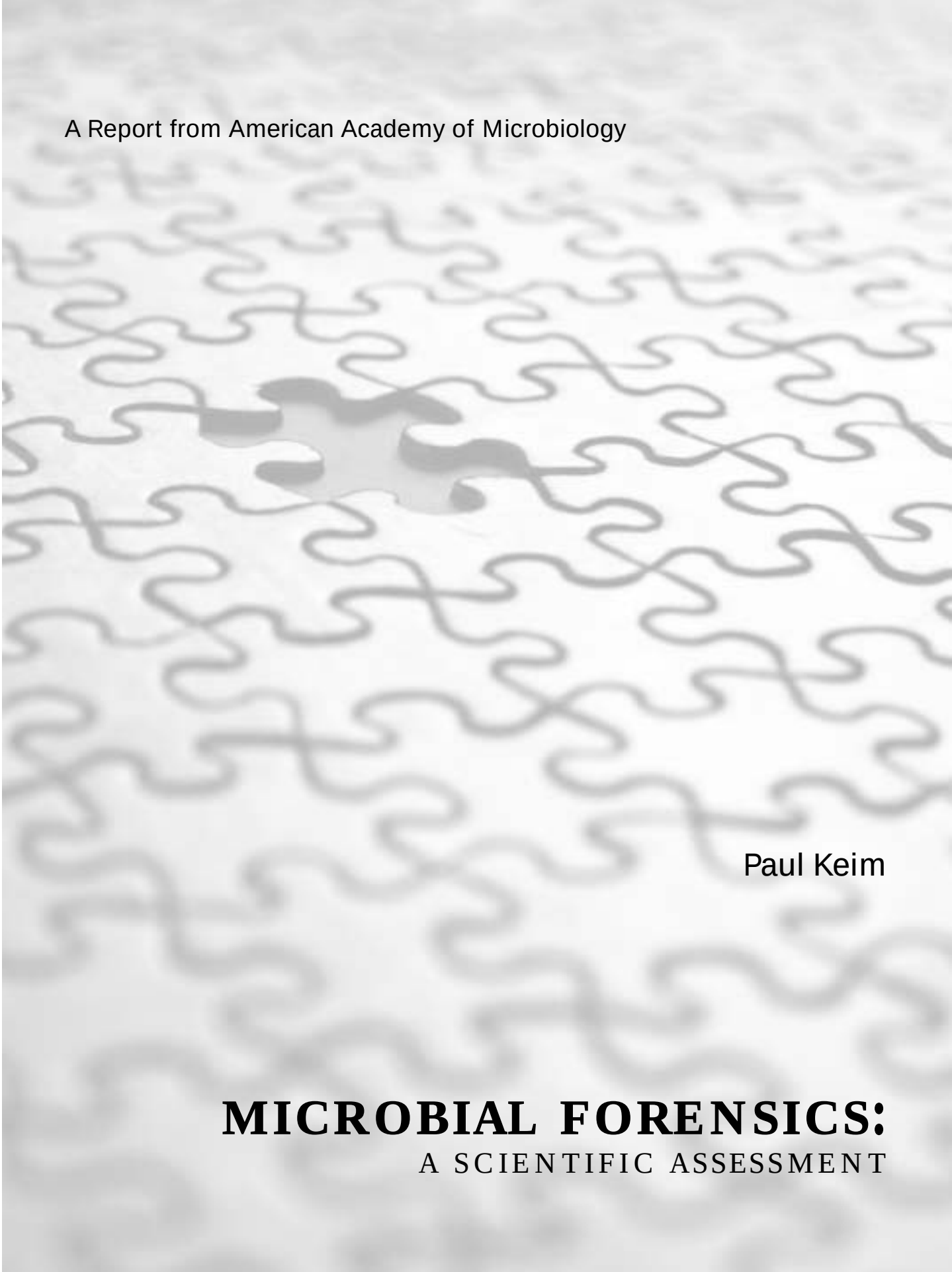

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EXECUTIVE SUMMARY

Microorganisms have been used as weapons in criminal acts, most recently highlighted by the terrorist attack using anthrax in the fall of 2001. Although such "biocrimes" are few compared with other crimes, these acts raise questions about the ability to provide forensic evidence for criminal prosecution that can be used to identify the source of the microorganisms used as a weapon and, more importantly, the perpetrator of the crime. Microbiologists traditionally investigate the sources of microorganisms in epidemiological investigations, but rarely have been asked to assist in criminal investigations. This colloquium examined the application of microbial forensics to assist in resolving biocrimes, with a focus on research and education needs to facilitate the use of microbial forensics in criminal investigations and the subsequent prosecution of biocrimes, including acts of bioterrorism.

A colloquium was convened by the American Academy of Microbiology and held in Burlington, Vermont on June 7-9, 2002. The purpose was to consider issues relating to microbial forensics, which included a detailed identification of a microorganism used in a bioattack and analysis of such a microorganism and related materials to identify its forensically meaningful source—the perpetrators of the bioattack.

Developing systems and methods to detect and track bioattacks will lead to greater safety and security for our nation against international terrorists. But it will also benefit the investigation of all biocrimes, including those carried out in a personal manner. In a very fundamental way, biocrimes are a public health concern and, as such, involve the public health infrastructure. Biocrimes against agriculture and the food supply system, in addition to impacting economic and political stability, have had and will continue to have consequences for human health. Partnerships among the law enforcement, public health, and agricultural communities could lead to long-term programs that will enhance efforts.

First responders to any suspected terrorist bioattack or other biocrime face a number of issues, beginning with their own safety and the safety of the public. After the primary issues of health and safety are addressed, they must consider forensic issues, such as proper collection of samples to allow for optimal laboratory testing, which is paramount, along with maintaining a chain of custody that will support eventual prosecution. Because a biocrime may not be immediately apparent, a linkage must be made between routine diagnosis, epidemiological investigation, and criminal investigation. There is a need for establishing standard operating procedures and training of first responders to meet these initial challenges, or, at a minimum, to be aware of them to minimize disturbance of the evidence.

While epidemiology and forensics are similar sciences with similar goals when applied to biocrimes, forensics has additional and more stringent requirements. Maintaining a chain of custody on evidentiary samples is one example of an extra requirement imposed on an investigation of a biocrime. Another issue is the intent in microbial forensics to identify a bioattack organism in greatest detail. If possible, forensic investigations will strive to identify the precise strain and substrain, rather than just to the species level, which might be sufficient in an epidemiological investigation. Some pathogen attributes that are unimportant in protecting public health may provide clues in a forensic investigation.

Although multiple national and international groups have developed lists of bioterrorism target pathogens, these lists are too narrow. An expansion of microorganisms relevant to food and water threats should be considered. Potential pathogens that could be used in a biocrime should be periodically reviewed to keep target lists current with scientific and political realities. Pathogenic potential, degree of protection (e.g., vaccination, effective therapy), and accessibility are a few of the characteristics that could be used to prioritize pathogen lists.

Computerized networks should be established to track infectious disease outbreaks in real time. Such systems do exist to some degree but better connectivity or communication is needed. These systems could alert public health and agricultural officials to the existence of a potential bioattack earlier than simply waiting for a report of a suspicious cluster of similar patients. Indeed, bioattacks might go undetected altogether if dispersed cases are not linked via such a system.

Once a biocrime is suspected, a wide variety of methods are available to identify the microorganism used in the bioattack and to analyze features that might lead to the source of the event (e. g., strain typing). A multipronged approach to such an investigation may be preferable, using many available methods—ranging from genomics to sequencing to physiology to analysis of substances in the sample. Infective samples are comprised of more than just the pathogen; analysis of contaminating spores or pollen, growth medium constituents, or even the water in the sample could be informative. Unfortunately, unique and possibly engineered pathogen attributes that enhance pathogenicity have to be considered for biocrimes. The needs of each case will dictate what tests may be needed.

Microbial forensics will be most effective if there is sufficient basic scientific information concerning microbial

genetics, evolution, physiology, and ecology. Simply studying the pathogen without understanding biotic and abiotic environmental backgrounds will lead to false confidence in our ability to detect it. Strain subtyping analysis will be difficult to interpret if we do not understand some of the basic evolutionary mechanisms and population diversity of pathogens. Phenotypic features associated with evidentiary pathogens also may provide investigative leads, but full exploitation of these features can only be accomplished if we understand basic principles that control microbial physiology. Novel technologies and basic research support are needed for many microbial forensic endeavors.

Additional microbial forensics information may be gained through analysis of the host response. This may be as simple as testing for humoral immune response by temporal IGM and IGG responses to the epitopes of the offending microbe, which may lead to a differentiating perpetrator from a victim profile. Assays may evolve to validated cellular responses and possibly microarrays.

High quality assurance and quality control standards for microbial forensics will ensure highly reliable results and those results that will stand up in a court of law. More importantly, the Quality Assurance/Quality Control practices will provide the public a degree of confidence. Standard operating procedures, training of technologists, proficiency testing, enhanced databases, and multiple analyses are some of the steps that will meet this need. Setting up a multi-tiered laboratory system, analogous to or building upon the Laboratory Response Network, would be a great help in the microbial forensic area.

Finally, the more precise and refined a microbial forensic system becomes, the more proper guidelines for handling and storage will be defined. Thus, improper dissemination or use of the pathogens will be reduced and inadvertent release will be minimized. An additional outcome of establishing these guidelines or rules is that the legitimate investigator will be protected to pursue research without unnecessary intrusion.

Colloquium participants identified a variety of needs and directions in the following areas: sample handling and collection, detection, research direction, data access, QA/QC, and education. General recommendations are provided for direction or insight for the scientific community, law enforcement community, legal community, and the public.

RECOMMENDATIONS

EVIDENCE GATHERING

- Establish permanent, cross-discipline communication and education programs.
- Identify and educate first responders.
- Establish a permanent team of microbiological experts as consultants to law enforcement.
- Develop standard operating procedures to avoid compromise of samples.

IDENTIFICATION OF BIOCRIME ORGANISMS

- Set up national tracking computer network.
- Develop new and standardize existing tests and diagnostic kits.
- Increase support for environmental microbiology and microbial ecology to enhance detection and identification of microorganisms from natural communities.
- Establish databases for intrinsic background species and biothreat strains for important and relevant geographical regions.

TRACING THE SOURCE OF THE ORGANISMS

- Develop new identification assays and physical analysis methods.
- Encourage research to examine microbial cell composition and whether some free-living bacteria from soil and aquatic environments are endemic, as well as microbial physiology.
- Develop new technologies to advance microbial forensics.
- Perform a more complete genomic sequencing on a minimum of three strains within the agent species and one close relative. In addition, the highest priority pathogens should have additional strains—perhaps 10 to 20—sequenced for signature development and to understand biological variation.
- Broaden the listing and prioritization of potential biothreat pathogens by an interagency group, with input from the scientific community.
- Establish a National Strain Repository to conserve reference material. This should be rapidly accessible to registered research programs.

THE INVESTIGATION

- Form a working group to evaluate, approve, and help implement appropriate Quality Assurance/Quality Control procedures.
- Establish a panel of microbiology and forensics experts for rapid peer review and validation before court cases.
- Adopt proficiency and validation testing to estimate false-positive and false-negatives rates.

EDUCATION, TRAINING, AND COMMUNICATION

- Establish a professional certification program in microbial forensics.
- Develop first responder training programs, as well as programs about biocrimes for public officials, law enforcement officers, and members of the judicial system.
- Consider a new journal or sections in existing journals on microbial forensics/biothreat response.
- Develop a public education program on the facts about biocrimes.

INTRODUCTION

The possibility of a criminal action using a biological pathogen has been known, appreciated, and well documented for some time. However, apprehension over the use of biothreat agents has greatly intensified after September 11 and October 2001, when cases of inhalational anthrax came to the forefront. In the "anthrax letters" incident, more than 20 persons developed this rare disease and at least five died. Many more were exposed and at risk. These cases were immediately seen as unusual and suspicious by the public health and law enforcement communities, because of the disease's rarity and distribution across four states in three noncontiguous regions. Investigations were immediately and intensely pursued. It was quickly established that these anthrax cases were contracted from opening or handling letters that contained anthrax-spore powder. While the letters had been sent through the U.S. postal service to specific targets, postal workers and others became infected secondarily. This was the largest impacting biocrime to have occurred in the United States.

Biocrimes require a unique governmental response involving coordinated efforts of both public health and law enforcement officials. While not identical in method or approach, the goals of these two efforts are similar. In both biocrime forensic and epidemiological investigations, the primary concern is that the infectious source is identified and more importantly controlled.

Infectious material, disease patterns, and case(s) characteristics are analyzed to generate insights into the event(s). While the event epidemiology can be satisfied with control and disease prevention, law enforcement must consider eventual prosecution and presentation of evidence in the courts.

During the anthrax attack, a number of questions became paramount, some of which were:

- Were cases in the four states related?
- Were they all caused by the same strain of the pathogen?
- Were all spore-bearing letters sent by the same person(s)?
- Who was the individual or individuals responsible for the murders?
- Did this bioterrorist threat represent an international or domestic incident?

Extending the investigation of an infectious outbreak beyond public health, as these questions clearly do, is a purview of microbial forensics. A forensic investigation focuses on the individual or group who perpetrated the threat. When a biocrime takes place, it is important not only to identify the pathogen involved, but also to trace the microorganism to its source—the perpetrator(s) of the bioattack and how the crime was carried out. Identifying the criminal(s) is obviously necessary for purposes of criminal justice and to prevent further attacks. It also needs to be quickly accomplished, if possible, to reduce fear and panic in the public. Although the number of persons infected by the October 2001 anthrax mailings was not great, over the ensuing months the attack was extremely disruptive upon our society and economy. It is clear that even minor bioterrorism events have the power to disable large societal infrastructures, overload laboratory facilities, and severely challenge personnel charged with managing and containing the bioattack. Indeed, the bioterrorism ripple effect far surpasses its initial impact, approaching the consequences of far more devastating initial events as transportation, communication, and commerce are seriously compromised. However, biocrimes are much broader than just bioterrorist acts and could be directed against individuals, institutions, livestock, and crops. The breadth of possibilities is frightening and will challenge our capacity to prepare.

Determining that the anthrax event was a biocrime was relatively straightforward; however, attacks on agriculture or the food supply system pose additional challenges. Many times it is quite difficult to determine whether the outbreak was a naturally occurring outbreak or an intentional event. For example, when public health officials conducted an epidemiologic investigation of *Salmonella enteritis* in Oregon in 1984, it was concluded that the outbreak was a food safety issue. It was only after a member of the Rajnesshee cult came forward that the event was recognized as a biocrime. Although unlikely to be intentional acts, the sources of the Foot and Mouth outbreak in Taiwan in 1997 and the recent West Nile Virus in the U.S. remain undetermined. The capability to identify the perpetrator is important to stop further criminal actions and also to protect legitimate researchers and medical personnel.

During a biocrime criminal investigation, microbial forensics will require and use traditional investigative methodologies, established molecular techniques, and newer advanced methods that still may be under development. Some existing methods include phenotyping, phage typing, fatty acid composition, and nucleic acid fingerprinting. Newer and developing technologies that will be important include use of genomic databases, microarrays, proteomics, isotope analysis, media characteristics, and bioinformatic analyses to interpret data in the appropriate context, to name a few. We expect the emerging discipline of microbial forensics to evolve, as new methods are developed and tested, while traditional methods are re-evaluated.

Tools from public health will contribute to a forensic investigation. In turn, knowledge, skills, tools, and experience honed in forensic work will enhance the public health foundation. Synergy between public health and microbial forensics already has been demonstrated with the current epidemic of West Nile virus, which is spreading naturally and not as a result of bioterrorism. Public health officials have been using the outbreak of West Nile virus in the southern U.S. to practice responding to diseases that are unfamiliar to most doctors and that might be spread in an attack

Attacks with microorganisms have been and will continue to be a threat to public health, societal morale, economic security, and political stability. Not only attacks directly against humans, but also use of microorganisms against agricultural targets—livestock, crops, and the food supply—can be included under the heading of biocrimes or bioterrorism. The impact of an attack on U.S. agriculture is highlighted by the fact that agriculture constitutes one-sixth of the U.S. gross domestic product—over a trillion dollars a year. The food and agriculture sector is the nation's largest employer; one of eight Americans works in an occupation directly supported by food production. Agriculture exports total over $50 billion annually, making the farm sector the largest positive contributor to the national trade balance. Officials are beginning to recognize that this vast network of food and fiber production, processing, distribution, and sales is a potential—even inevitable—target of hostile interests employing biological agents for political, economic, or criminal objectives. Even the threat of attack could jeopardize consumer confidence, disrupt commodity markets, and wreak economic havoc.

EVIDENCE GATHERING AT A SUSPECTED BIOCRIME SCENE

Experience with biocrimes to date, including hoaxes, has shown that the scene of a biocrime may be a hectic, relatively small area filled with many people trying to protect the safety of individuals while gathering and transmitting information. Still, samples must be collected in an organized manner, avoiding contamination, and maintaining viability and the chain-of-custody.

It is crucial to establish communication linkages between the public health and law enforcement communities. Law enforcement officials need to understand what is normal in medical and epidemiological investigation procedure. The first responders to an investigation scene need to know what safety precautions to take to avoid infection, the spread of contamination, and to preserve the crime scene. Public health personnel need to understand what additional steps are required when a public health incident becomes a criminal investigation (e.g., preservation of evidence). Simple requirements, such as evidence preservation, can be accommodated by establishing better communication among these groups.

Recommendation: Establish permanent communication and cross-discipline education programs for public health and law enforcement communities.

Special emphasis should be placed on cross-discipline training for effective action at the site of a biocrime. This will involve identifying and training local personnel who will be first responders in crime scene requirements, responsibilities, and their role in any investigation. Standardized training should also include self-protection, methods on not compromising a site, and using identification kits.

Recommendation: Some first responders should be identified in each community and standardized forensic specific training initiated.

Another measure that will increase the probability of a successful biocrime investigation is trained teams of investigators established in advance. These teams should be multidisciplinary in composition—public health personnel, laboratory scientists, and other necessary specialists, particularly pathogen-specific experts and statistics/phylogenetic experts. While it may be impossible to anticipate every contingency, a panel would also be capable of determining team weakness for a specific event and be able to recommend recruitment of additional experts. Legal experts and law enforcement officers could either be members of the panels or available as consultants to facilitate communication with law enforcement agencies. Prior to an

event, these expert teams would be responsible for reviewing the literature, writing articles on current pathogen knowledge, developing guideline recommendations, and providing oversight for development and implementation of Standard Operating Procedures on how to handle biocrime scene samples. In the initial days following a biocrime crisis, panel members could provide quality scientific evaluation until more specific and long-term expertise is identified and put into place.

Recommendation: Establish a team of experts who understand the biology of organisms that are likely to be used in a bioterrorist attack and who could be called upon in a crisis. These individuals could review past events to improve processes.

In support of trained personnel who would be in charge of a biocrime site, it would be helpful to have mobile laboratories and related equipment to do basic microbiology and forensic analyses at the scene. Some functions that could be provided would be the capability for quickly initiating state-of-the-art analysis, microscopy, shower-in-shower-out logistics, sterilization, and autoclaving. At the core of the investigating team's job will be to obtain samples both from victims and the environment to identify the biothreat organism, to identify the source of the bioattack, and to preserve evidence for a trial. An intermediate goal will be to physically localize the source of the biocrime at the site of exposure—from food or water, through an air circulation system, or, as in the anthrax bioattack, from mail.

Since it may not always be clear what the route of exposure was, samples should include background/ambient/environmental material. To establish "normal" ambient levels of organisms, sampling of areas peripheral to the scene should be performed. To help determine the route of infection, collection of samples must be done in an organized manner. As at an archeological dig, a grid of the biocrime site can be created and samples organized within this framework. Sample collection protocols would benefit from standards developed by the National Committee on Clinical Laboratory Standards (NCCLS) or other agencies with relevant or appropriate expertise. Expert information on collecting samples from a variety of environmental sources could contribute to the design of the protocols. Protocols for collecting clinical samples from animals are available from the United States Department of Agriculture (USDA) National Veterinary Services Laboratories in Plum Island, New York, and Ames, Iowa. Protocols for sampling contaminated food are available from the Microbial Outbreaks and Special Projects Branch of the USDA Food Safety Inspection Service in Athens, Georgia, and from the Food and Drug Administration (FDA). Plant specimen collection protocols are available from the USDA's APHIS Plant Protection and Quarantine. Protocols for collecting samples from soil are still needed.

At all times, persons collecting specimens must be aware of maintaining the chain-of-custody to preserve validity for subsequent criminal proceedings. One model should be followed; we recommend those standards set by the Federal Bureau of Investigation (FBI). Samples collected at the site of a biocrime should be assigned barcoded sample labeling, if possible, with information immediately entered into a database. Access to such databases for both entry and editing must be controlled to monitor errors and to ensure the sample chain-of-custody.

Another step in preserving the chain-of-custody is to place samples into tamper-proof, secure containers. The use of tamper-evident specimen bags is recommended, as is done routinely in other forensic practices. Specimens should be transmitted in safe packaging to the appropriate laboratory for testing.

Properly storing all evidentiary materials is imperative. Some samples may degrade during room temperature storage or freeze-thaw procedures. Lastly, all original material should be retained, including secondary material.

Recommendation: Develop standard operating procedures for sample collection, documentation, and access, storage, and transmittal so that samples are not compromised.

IDENTIFYING AN ORGANISM USED IN A BIOCRIME

In the forensic arena, precise and robust pathogen identification may be crucial to the eventual prosecution of criminals.

TOOLS FOR IDENTIFYING THE ORGANISM USED IN A BIOCRIME

Samples from the site of a biocrime may be analyzed on-site or in a laboratory. Identification may be facilitated by establishing a national computerized network to track outbreaks of infectious diseases (e.g., PulseNet) and to report unusual and usual symptoms. Also, the data in existing networks (e.g., Promed) may be needed for evaluation. Even unusual patterns of typical flu-like symptoms could be important, since most bioterrorism agents present with flu-like symptoms. Such a computerized network may alert authorities to the occurrence of a biocrime earlier than if recognition of a cluster of clinical cases is used to signal the event. Moreover, data collected from the network and processed in the

computer can provide clues as to what organism might be involved in a biocrime once it is suspected.

Databases of this type already exist, notably in the field of pesticide-related illnesses, which have to be reported by law, and for plant diseases. In the human infectious disease field, the PulseNet surveillance system for food-borne pathogens, which was established primarily to determine the prevalence of food associated illness, could potentially be expanded to detect an outbreak that is a single source or could at least be used as a model for such a network.

Recommendation: Set up a national computerized network to track disease and unusual symptoms for humans, as well as animals, plants, food, and water.

When monitoring for a bioterrorism event, it will be important to keep in mind the potential for bioattacks against agriculture—crops or livestock. Agriculture could be seriously damaged by a slow expansion of a pathogen that first is not detected and becomes established. Even if prepared to identify the pathogen, it may have been bio-engineered to be more devastating or simply more effectively disseminated. Monitoring and analysis systems need to be developed to identify unusual patterns. In addition, there is need for tests that are quantitative (to allow investigators to "swim upstream" to the source).

Identification of a biocrime agent might be done with pre-made kits, at least for the most likely pathogens. At the present time, the quality of diagnostic kits is mixed and is in great need for further systematic evaluation. Currently, biothreat kits do not have validation, standards for training, nor are they required to go through a systematic evaluation. Therefore, kits need to be standardized along with qualitative and quantitative evaluation. Multiplex kits would be particularly valuable, with validation and sensitivity and specificity established, and possibly a certification program. Tests should be easy, reliable, and directed against regions of genes known to be important for toxin activity (to get around the "Trojan horse" deception). Multiple test types should be developed to avoid problems of inhibition (deliberate or by chance) that could give false negatives.

Recommendation: Tests/kits should be standardized—sensitivity and specificity need to be established and training standardized at the federal, state, and local level. Blinded comparison of kits should be done.

Opportunities for field validation must be made available to the private sector to encourage their active participation in development efforts. Such exercises permit fair competition among technologies, which is superior to reliance upon "governmental insider" systems. To stimulate private sector participation, appropriate sequence and appropriate, but not compromising, data from other databases must be available to the private sector—perhaps through collaboration with government and with appropriate clearance. Because market forces may not be sufficient, stimulus, research, and development funds should be provided and widely publicized for recruiting company participation. Finally, corporate participation will require market guarantees upon development and testing of valuable technologies.

Recommendation: Government sources of funding are needed to encourage companies to develop pathogen identification kits. Regulations to fast-track assay development through the FDA review process should be considered.

A number of methods are currently available that would be useful in identifying a biothreat agent used in a biocrime. Classical phenotypic assays for physiological properties are among the most basic. Other methods include:

- Sequencing of DNA/RNA in samples and genomic sequencing of culture isolates.
- Determining phylogenetic patterns of single nucleotide polymorphisms (SNPs).
- Collection and dissemination of genomic information.
- Association of microorganism genotypes with phenotypes.
- Pathogenicity arrays (including 16S rRNA probes) to detect artificially constructed hybrid microorganisms.
- Screening tests for detection of antimicrobial resistance markers.

Use of multiple test methods is desirable to avoid misidentification of agents caused by induced or engineered mutations. To this end, portions of samples should be saved for additional investigation or confirmatory testing. Blind, barcoded sample replicates (e.g., 10% of the replicates) are recommended.

Recommendation: Multiple methods should be used for identification. Split samples, when sufficient material is available, and independent laboratory analyses should be used if possible. Samples should be held in the event there is controversy about findings.

The utility of DNA hybridization microarrays will be great. Microarrays, which can hold up to 400,000 probes per chip, have recently become available for many bacterial analyses. Microarrays could play a role in identifying bioterrorism pathogens in some circumstances, but may have some limitations. One requirement is that the microarrays would need to be sufficiently sensitive and contain probes for key

microbial genetic components (e.g., toxins, virulence factors, antimicrobial resistance markers). In addition, they will have to be cost effective and minimize operator error. Protein microarrays could be of value, but may be prone to loss of sensitivity due to conformational changes that occur during attachment to chips. An acceptable and documented level of reproducibility and discrimination must be reached before these are adopted.

Expression arrays may be useful as a preliminary characterization of overall phenotype; however, we do not see an important role for expression arrays in forensics in terms of strain identification. Their value is limited by confusion caused by expression plasticity, evolutionary convergence, and pleiotropic effects. For expression arrays to work, standardized growth conditions must be defined so that all isolates have the same culture history.

Databanks containing complete or partial sequences of potential bioterrorism agents could also be a valuable resource in the event of a biocrime. Complete, as well as partial, genomic sequences should be maintained. All available information, including raw sequence reads, should be accessible so that the best resolution possible can be attained for forensic identity. In addition, the cost-benefit ratios of the different sequencing approaches need to be considered. Partial sequences may have to suffice early on in the investigation, especially if there are large numbers of isolates to be tested. Partial sequences can be adequate at preliminary stages and to help guide targets of polymorphic value. With respect to differentiation of the organism, only a few SNPs (5-10) may be needed for species identification (e.g., to distinguish *B. anthracis* from *B. cereus*), but the number of SNPs will depend on the diversity of the genus/species and upon its phylogenetic structure pathogens. Draft sequencing will have higher error rates, and differences will have to be confirmed via subsequent and alternative methods. Raw sequence reads will assist in identification of true differences from sequencing errors.

Sequencing costs are declining each year due to incremental improvements in technology. Additionally, there is the promise of "post-Sanger" sequencing methodologies that may dramatically increase throughput in the near future. For these reasons, whole genome sequencing is likely to have increasing value as a routine microbial forensic tool. Using whole genome sequencing, or high-density SNP analysis, investigators can also learn about possible phenotypes of the strain, how similar the strain is to other, previously typed genomes, and whether it is likely the strain contains engineered sequences (and what they may be). In the same manner, "whole-community" sequencing methodologies, where whole DNA from environments is sequenced rather than individual organisms, will likely also become important, for both identification of the microbe in the contaminating sample and for subtyping the microbe.

If a genome sequence is needed, draft sequencing (about 90% coverage) may be sufficient in some circumstances. Pre-attack, species-specific, or virulent clone-specific entire genome sequences should be obtained to identify unique signature regions of the genome. Post-attack, entire genome sequence may not be needed if unique signature regions of the genome are known and strain-to-strain variability is not great. However, in some cases the number of strains that need to be sequenced will not be known *a priori*, and each organism will differ in how many need to be sequenced with current methodology.

Two problems potentially affect the utility of genomic signatures. First, non-proprietary and open databases of genomic sequence-based microorganism signatures have distinct advantages over proprietary or closed databases. Widespread examination and validation of the databases enable better review and utility to develop assays. The robustness of closed databases may be suspect, or at least unknown, due to limited scrutiny. Second, it will be important to avoid limiting agent signatures to specific technologies. Technological advancement in this area is extremely dynamic and, thus, platform inflexibility over time may ordain an assay to obsolescence.

Laboratory capacity to generate and compare genome sequences could also become an issue. Regulatory permits and certification should be considered for existing "qualified" genome sequencing laboratories prior to a bioterrorism incident. The addition of more genome sequencing laboratories to a microbial forensic network would be helpful to ensure surge capacity for periods of high demand. Certified laboratories should have access to all biothreat agent genome databases in anticipation of an eventual event. This information flow will have the ancillary benefit of encouraging the advancement of our knowledge of microorganism evolution.

Identification of the source of a microorganism used in a biocrime will be facilitated by consolidation of existing databases of genomic sequences and their expansion through sequencing of additional selected strains. Revised prioritization of potential pathogens lists may contribute to the selection of strains to be sequenced. Open access to databases should be the norm; open access may enhance security, rather than compromise it. Western scientific endeavors feed off free information flow and ideas. Shackling valid researchers should be done with great care to avoid crippling the effort. Errors will inevitably creep into databases, and, while

they may not be completely eliminated, efforts should be made to estimate their levels, understand their sources, and control their reoccurrence.

Recommendation: Genomic sequence and other databases should be maintained with open access to stimulate research and to increase accountability, whenever possible.

Environmental DNA extraction is one technical challenge that needs to be addressed. Extracting DNA from samples at a biocrime site may be complicated by unknown and unanticipated contaminants and inhibitors. Extraction may also be difficult if a biocrime takes place in an outdoor environment where soil, mud, or nutrient-rich water (such as in a pond or swamp) is the matrix being examined. Promising work in the area of environmental microbiology suggests many solutions to these problems, and scientists working in this field should be consulted when developing forensic methods.

Recommendation: Increase support for environmental microbiology and microbial ecology to improve current methods for the detection and identification of the microbiota from natural communities. These techniques and approaches may prove useful for the identification of biocrime agents.

It is necessary to discriminate intentional criminal use of a biothreat agent from natural disease occurrence. Clearly the presence of some pathogens automatically will raise suspicions, e.g., any case of smallpox will be automatically considered suspicious and investigated rigorously as a biocrime. Likewise, a more common disease that occurs outside of its normal environment will also be considered suspicious and rigorously investigated (e.g., anthrax in the eastern U.S.). Disease patterns can be more precisely examined using comparative genomic analyses of species and strains to establish regional background expectations. The occurrence of a naturally rare—but in the laboratory—biothreat strain would be one approach to biocrime recognition. Genome signature regions from a suspected bioterrorism isolate can be compared with those of "wild-type" background strains, if the prior studies have been performed. Databases containing these signatures must be available and well populated with data for strain analysis and the comparative genomic approach to be successful.

Recommendation: Establish databases for intrinsic background species and biothreat strains for important and relevant geographical regions.

TRACING THE SOURCE OF THE ORGANISM

When an infectious disease outbreak occurs, and a determination has been made that it is not a natural outbreak, but, instead, represents a biocrime, identifying its source—the person or group who perpetrated the biocrime—will become an urgent priority. Exclusion of the innocent is crucial and one of the more powerful conclusions that is accomplished during a forensic investigation. In the case of biocrimes, it is obviously important to protect innocent individuals' rights. In addition, many of the experts who can greatly assist in a biocrime investigation will be potential suspects due to their unique qualifications. Excluding them from the suspect list may be necessary prior to their recruitment and a new aspect of source attribution.

Identifying the source of a biocrime may require gathering information about both the organism itself and about the matrix in which it is found. Many current techniques are available to carry out this task, with new ones under development. Timely validation of new techniques will be important

METHODS FOR IDENTIFYING THE SOURCE OF A MICROORGANISM USED IN A BIOCRIME

Identifying the source of a biocrime by analyzing the bioattack agent and its milieu can be a productive approach. In general, a multi-pronged strategy should be adopted. Attention should include attributes that are consistently present and not liable to be lost during storage.

Many features of the microorganism itself will be useful in identifying the source of a bioattack. Genomic sequence signatures should be searched in established databases. Signature DNA sequences can help to identify specific strains. Proteomic signatures can also be informative. Physical attributes acquired by microorganisms during preparation, such as during weaponization, can provide clues. Isotope ratios (^{13}C and ^{15}N) can be used to determine the age and source of microorganisms. Biomarkers can be utilized to identify microorganisms used in industry or by authorized laboratories. Pharmacogenomic data may be useful in identifying the source. Traditional physiologic methods—fatty acid composition, phage typing, and serotyping—also may provide useful information.

More advanced assays that are currently available for identification of potential pathogenic organisms include FISH, TAQMAN, other amplification methods, and microchip analyses.

Recommendation: Further development of identification assays is needed so that more specific and robust assays will be available.

Features of the material in which the microorganism is found can also be useful in identifying the source. Remnants of growth media adhering to microorganisms may help determine the source. For example, one attribute may be the source of serum used to grow the microorganism, such as camel or kangaroo serum. Minor culture medium components associated with microorganisms may identify the location or conditions used to generate the biocrime material. Pollen contamination analysis is one example of this tactic. It may also be helpful to analyze spore coats when investigating possible exposure to spore-forming bacteria. Analyzing matrix components of samples in which microorganisms were detected may also be productive.

Recommendation: Encourage better physical analysis methods to identify sources of culture material (isotopes and other physical chemical features), as well as ways to identify sources using pollen, fungi, or other environmental features that might yield geographic or temporal information.

Bacterial endemism (i.e., the existence of unique strains of bacteria at only one locale on Earth) may be important for source attribution, but it is not well understood. Bioterrorism agent samples might be contaminated by other bacteria that can be traced to a particular production location—that is, assuming we know that some bacteria are endemic. Currently there is very little evidence of endemism in bacteria; however, most microbiologists believe that it exists at the strain level and increasingly evidence supports this view. This is a very significant new area of research that needs more funding.

Recommendation: Funding should be provided for studies in several emerging areas that show great potential:

- Gather information from exhaustive microbial surveys to understand better where microorganisms are naturally found. Determination of background levels provides a better understanding of microbial ecology.
- Microbial cell composition to determine if there are signatures of water sources, for instance, that may be used to identify origin.
- Determination if some free-living bacteria from soil and aquatic environments are endemic, that is, found only in certain locations on Earth versus being cosmopolitan.

DETECTING BIOENGINEERED FEATURES IN A MICROORGANISM USED IN A BIOCRIME

An especially important aspect of a microorganism used in a biocrime will be whether it has been bioengineeered. Bioengineering can make a common strain of a microorganism particularly dangerous. In addition, such "Trojan horse" strains can be more difficult to recognize. On the other hand, detecting bioengineered properties can provide clues to the microorganism's source.

Placing a toxin gene into a commensal organism is a fairly simple procedure that illustrates both the enhanced danger and the potential deception associated with bioengineering. For example, a bioterrorist could insert the gene for cholera toxin production into a nonpathogenic *E. coli*. If this *E. coli* could then produce the cholera toxin *in vivo*, a municipal food or water supply contaminated with such an organism could cause a widespread outbreak of cholera. Stool cultures from the victims would yield growth of the *E. coli*, but it would at first analysis be part of the normal enteric flora. Crucial time could elapse before investigators realized that the *E. coli* was in fact the culprit. This example is not unimaginable, since similar things have already happened in nature. For instance, the heat-labile toxin produced by enterotoxigenic *E. coli* (the cause of travelers' diarrhea) is very similar to cholera toxin. The Shiga-like toxins produced by *E. coli* O157:H7 and other serotypes are virtually identical to the toxins produced by *Shigella dysenteriae* (the cause of bacillary dysentery). Thus, identification of the biothreat agent must be more comprehensive to quickly screen for potential engineering, such as insertion of virulence genes, vector fragments, drug resistance mutations/genes, or genes for toxins.

DEVELOPING TOOLS FOR IDENTIFYING THE SOURCE OF A MICROORGANISM USED IN A BIOCRIME

Newer and more rapid sequencing capabilities that reduce the time to obtain results are constantly being devised. Genomic tools that enable the tracing of microbial evolution can narrow a list of possible sources. Rates of evolution may provide forensic information concerning timing—both about culture history and epidemiological dynamic, or timing of infection. It is important to better understand population dynamics and phylogenetic analyses for identification of the source of biocrime organisms.

One subtle way of differentiating strains of a microorganism, and thus pointing to one source rather than another, is by monitoring or measuring changes in host response to pathogens. Many genes are up regulated in such a situation, such as immune response genes. Microarrays would be good for this complex task. Mass spectrometry approaches may be useful as well. Simple measurements of the time-tested humoral response (IgM and IgG) may the most reliable.

Recommendation: Support increased research on microbial physiology. Research will be needed to determine the effect of growth conditions/mixed cell cultures on gene expression, post-translational modifications, etc.

A more futuristic approach is nanotechnology that could enable miniaturized, disposable assays. In the same category is enhanced detection of microbial agents by continuous reagentless environmental monitoring systems that can trigger collection of larger samples when positive. "Sniffers" and acoustic resonator devices to detect the presence of microorganisms are available, but not yet validated.

To speed availability of these new and emerging tools, funding agencies should offer programs to encourage development of new tools. Validation will be requisite before new tools are released for widespread use. Availability of standardized criteria for validation of new tools is desirable. At this time, the question of who is responsible for the validation is unresolved.

Confidence in new methods will be mandatory for their successful use in forensic settings. A number of steps—largely following the traditional route to scientific acceptance—will contribute to achieving the needed level of confidence. When results of a new technique are confirmed by the "gold standard" or by more than one independent method, the new method is on the road to being accepted. Clinical findings can also be used as part of the "gold standard" by which test results are validated; favorable outcomes in situations in which the new resources were used will increase confidence. Confidence is also engendered when, in the tiered system of clinical laboratories, higher-level laboratories confirm the tentative results of lower-level laboratories.

Recommendation: Develop and validate new technologies to further advance microbial forensics. New forensic methods should be published in peer-reviewed, reputable scientific journals. When security concerns are paramount, traditional peer review may need to be modified to accommodate both requirements.

GENERATING ADDITIONAL GENOMIC SEQUENCES AS A RESOURCE FOR IDENTIFYING THE SOURCE OF A MICROORGANISM USED IN A BIOCRIME

Much of the work in differentiating microbial strains to distinguish a source for a bioattack depends on comparison of genomic sequences. Obtaining the maximal benefit from this technique requires having more extensive collections of DNA sequences. New sequences are continually being added to existing databases, but an organized program of research will accelerate this process. Determining which additional genomes to sequence will be done on a case-by-case basis. The identity of new genome sequences to be developed depends on how well characterized pathogens are already and how diverse the genomes tend to be within a pathogenic species. Some entire genome sequences are needed, as well as measuring variation through comparative genomics.

In general, for any microorganism that is a potential bioterrorism agent, it would be desirable to sequence multiple strains of the same pathogen. These strains should span the known and suspected diversity within that pathogenic species or virus. One desirable approach would be to completely finish a genome sequence for at least one important strain to serve as the species reference. In addition, at least two additional genomes from the most diverse strain should be sequenced at the "draft" level. These within species comparisons will then dictate how many, which strains, and to what quality level additional within species sequencing should be performed. In addition, a close relative of the biothreat agent should be chosen and "finish" sequenced to serve as an outgroup. An outgroup comparison will help to identify species-specific signatures and lead to insights about virulence factors necessary for pathogenicity. This strategy is considered a minimal approach to bacterial genomic analysis, and additional strain or "finish" sequence may be necessary and should be decided on a case-by-case basis. For small viral genomes, more isolates with "finish" quality sequencing will be more appropriate. In contrast, larger eukaryotic-pathogen genomes may allow only draft sequencing of a single isolate due to their great cost.

Recommendation: Perform more complete genomic sequencing of all likely bioterrorism agents. As a minimum, three strains within the agent species and one close relative should be performed for bacterial pathogens.

RE-EXAMINING PRIORITIZATION OF PATHOGENS

Lists of potential bioterrorism agents have been available for some time. Currently existing are two lists, designated A and B, each containing eight microorganisms. Having priority lists is valuable; for one thing, it focuses research and stimulates funding for development of detection assays. We believe that more frequent re-appraisals of these lists should be undertaken. Re-appraisals must be cooperative efforts across domestic agencies within the U.S. and between countries and should pay greater attention to pathogens that can cause economic and agricultural damage, as well

as those that are direct threats to public health. Common foodborne pathogens (*Shigella* and *Salmonella*) have been previously used as agents in biocrimes, and any further prioritization lists must consider these a threat. The threat potential to agriculture is considerable, and such pathogens must be included.

Pathogens that are easily transmitted, accessible, durable, and cause disease that is debilitating or fatal should be considered for a higher priority rating. Agents for which there are no vaccines or therapy are also of importance.

Recommendation: Lists of potential biothreat pathogens should be broadened to include economic, agricultural, as well as public health, considerations. These lists should be reviewed periodically. The priority of animal, plant, and foodborne pathogens should be upgraded. An inter-agency group with input from the scientific community needs to be involved in prioritizing listings of pathogens potentially able to be used in a biocrime. While there may be a common core list, it is more likely each agency, by virtue of its mission, will have a specialized prioritized list.

CURRENT AND NEEDED DATABASES

We have already noted the fundamental importance to microbial forensics of databases containing DNA sequences of all potential bioterrorism agents. Many excellent genomic databases of possible biocrime agents already exist; however, they tend to be decentralized. At the present time, the FBI and the DOE/DHS are establishing the requirements, framework, and location of a DNA database that will contain all available genetic information and other assay potentials on these threat agents. (At the same time, the FBI is also determining the framework and criteria for a relational information database on individuals who have access to these threat agents and which agents they have access to.) We need more complete databases of sequences and other biological and chemical information on potential bioterrorism microorganisms, as discussed above.

With more complete sequence databases, it will be possible to develop an error rate based on whole genomes that will allow a *de facto* definition of species. Consulting this database will ideally make it possible to answer questions such as: Is this isolate *B. anthracis*? Is it this strain of *B. anthracis*? Did it come from this specific location? Depending on the question, we would like to have some level of confidence in our conclusions from the data. It is not yet clear how many complete or partial genomes will need to be sequenced to achieve this goal.

Recommendation: Establish pathogen databases based on nucleic acid, DNA/RNA profiles, proteomics, and other phenotypic properties. The highest priority pathogens should have additional strains—perhaps 10 to 20—sequenced for signature development and to understand biological variation.

In addition to more complete databases of genomic sequences, we need a national pathogen repository, along with the expertise to manage it. The American Type Culture Collection (ATCC) micro-repository has stopped supplying select agents due to legal liability; the U.S. military will not supply them due to self-imposed restriction; and public laboratories are overwhelmed with requests and additional federal regulatory oversight. Providing samples of reference strains of biothreat microorganisms brings up issues of access, security, and culpability for future biocrimes. Yet, access to biothreat strains is essential to our national defense effort. A central repository for live cultures and inactivated biological material should be established that would be provided rapidly with minimal charges. Increases in research efforts will not be realized if insufficient reference material is not available.

Recommendation: Establish a National Strain Repository to conserve reference material and rapidly provide the material to registered research programs.

Persistent questions that will arise as databases are created and/or enlarged include: How open should such databases be? Who should have access to the information they contain? At the present time, there are barriers to access to some databases, such as classified work and pharmaceutical data. While the optimal degree of access may vary from one database to another, in general, we advocate the principle of "the more open, the better."

Some specific guidelines concerning database access may include:

- General database access will accelerate our understanding of microbial biology and evolution; access may be more valuable than the risks associated with misuse.

- Raw genomic data should be open, though not necessarily the annotation (at least with respect to some agencies, because of diagnostic markers).

- PCR primers and diagnostic genomic regions may require restricted access. However, it must be recognized that such restrictions will inhibit diagnostic innovations.

- On a case-by-case basis, a panel of security and scientific experts should be convened to determined material sensitivity.

- There should be barriers to ongoing forensic investigation material.

- There should not be barriers to access for non-bioterrorism microorganisms.

- Database contents can be demanded by both sides during legal proceedings, so the amount of information in any database that is made public should be carefully determined.

- Information can be required from database users prior to granting them query privileges.

While acknowledging that national security is a paramount consideration, we believe that open exchange of information may more rapidly enhance security, by enabling development of detection methods and countermeasures to bioterrorism.

Recommendation: Proprietary and closed databases can be protected by electronic security measures and legal agreements among institutions. Access should be extended as broadly as possible within these requirements.

STATISTICAL AND PROBABILITY ANALYSIS

There is a need to develop statistical and probability approaches for pathogen and strain identification. While we cannot expect the same level of certainty as found with sexually reproducing organisms (e.g., as in human identification), ideally identification capacity should be defined for each set of microbial markers for the various biocrime agents. Initial determinations, at least, may be qualitative, but quantitative values will provide stronger analyses and more consistent with what the legal system has grown to expect. Analysis of nearest neighbors and background frequencies are the minimal data that are needed to establish these. Achieving the best estimates will require cross-disciplinary work between molecular biologists and microbial population geneticists using available data. While statistical analysis is highly desirable, with some types of investigations and analyses this may not be available. The lack of probability criteria for such analyses should not prohibit their use in a forensic context, but, rather, encourage their development.

Recommendation: The degree of certainty should be established for all technologies based upon statistical genetics concepts and principles.

FUNDAMENTAL STEPS NEEDED FOR PRODUCTIVE AND RELIABLE INVESTIGATIONS

Microbial forensics is essentially a laboratory activity. As such, the fundamental goal of microbial forensics must be accurate and valid results, just as in clinical laboratory work. While forensic requirements may add further criteria, at the first level the underpinning of any microbial forensic investigation is to meet those fundamental criteria that are intrinsic to any laboratory activity. Some of these requirements are: quality assurance/quality control (QA/QC) criteria; quantitative evaluation of the level of false-positive and false-negative results associated with various procedures; establishing confidence in all methods by rigorous validation steps accompanied by peer-reviewed publication; and maintaining a highly skilled workforce through regular proficiency testing. A high level of reliability in testing results can also be advanced by establishing a multi-tiered laboratory network system, in which more complex testing is performed by fewer and more-specialized laboratories. All of these considerations apply both to testing done to identify a microorganism used in a bioattack and to testing done to trace that microorganism to its source.

QA/QC STANDARDS

Several QA/QC criteria can be implemented that will establish confidence in the results of microbial forensic investigations. In the pre-analytic phase of testing, QA measures are needed that define proper collection, transport, and storage of samples. In the analytic phase of testing, QC procedures should be implemented that describe proper performance during testing. Also during the analytic phase, QA measures that identify proper controls should be adopted.

With regard to personnel qualifications, QA measures should define competency requirements of testing personnel. To identify and correct deficiencies, testing personnel must participate in proficiency testing programs and competency assessment activities.

When establishing QA/QC criteria, those developed by industry should be taken into account. Requiring that forensic laboratories comply with ISO-9000 standards or similar standards should be considered. In addition, it would be beneficial to set up a system of national certification (similar to the certification requirements under the Clinical Laboratory Improvement Act of 1988 (CLIA '88)) for laboratories involved in forensic microbial testing. Appropriate QA measures are needed to evaluate the interpretation of test results by individuals performing the tests or by individuals analyzing test results.

Recommendation: We consider adopting the FBI report on "Quality Assurance Standards for Forensic DNA Testing Laboratories" as a template for providing QA/QC guidelines for microbial forensics, as reviewed by state-level DNA committees and subcommittees. In addition a working panel should be formed to evaluate, approve, and help implement appropriate QA/QC procedures.

PEER REVIEW IN THE CONFIDENCE-GENERATING PROCESS

Peer review plays a well-established and fundamental role in the scientific process by reducing the likelihood that erroneous or misleading results or conclusions become accepted. In a microbial forensic investigation, too, stringent review can help to promote confidence in the results, both by the public and by judicial bodies.

The best examples of peer review are at the level of scientific journals, as well as presentation at scientific meetings. However, these traditional peer review venues are not applicable to every aspect of standard operating procedure development or to the interpretative process. For instance, database details may not be available for traditional peer review.

Validating results during an ongoing investigation—"validating on the fly"—will be especially problematic for traditional peer review mechanisms. Results of investigations will need to be validated eventually for forensic credibility. However, traditional procedures of peer review could be too time-consuming for a rapidly evolving investigation. A review panel of experienced experts may be considered to achieve peer review in the more dynamic setting of microbial forensics. This approach may be necessary and a better alternative than not using a technique for identifying a pathogenic agent and, hence, compromising public safety.

Scientific and forensic validity of results of ongoing investigations can also be enhanced by forethought and measures taken before an event actually occurs. There is a need to develop rapid tests for such investigations, as well as for proficiency testing to be implemented before urgent testing is needed. As with all microbial forensic laboratory work, standard QA/QC criteria can be instituted.

Recommendation: Establish a panel of microbiology and forensics experts for rapid peer review and validation before court cases.

FALSE-NEGATIVE AND FALSE-POSITIVE RESULTS

False-positive and false-negative results are inherent in many assays. To some extent, there is a trade-off between these two types of error. Whether a test should be skewed toward sensitivity or specificity depends on, for instance, the patient population in whom it is being applied and the goal of the test—whether it is for screening or confirmation. As long as the percentage of false positives and false negatives is kept very low and is known, and tests are used in appropriate settings, testing can be done reliably.

All of these considerations apply in equal importance to forensic testing as to clinical laboratory testing. In microbial forensics, early presumptive testing needs to be as broad and as rapid as possible, whereas the specific, forensic-level tests need to be as specific as possible. In addition, during early testing there must be a balance between attaining a rapid response capability and instituting checks to reduce false-positive findings, which may inadvertently create panic. Preliminary and incomplete analyses may have higher false positive results, and this needs to be estimated through validation studies and documented. Policy makers must be educated to understand better the issue of false-positive results and false negative results and their respective confidence levels.

In summary, microbial forensics testing in the field should minimize false-negative results (although they may be inevitable) that might delay the progress of an ongoing investigation and create a false sense of security for first responders. On the other hand, a certain level of initial false-positive results may need to be tolerated to guarantee low false-negative results. We recognize that false-positive results are not completely benign, as they may initiate the early stages of a response. This creates greater expense, inconvenience to those dependent on access to quarantined areas, and potential media frenzy. In addition, credibility and the confidence of the public and policy makers could be damaged by issuing false positive results. To compensate for this inflexibility, two (or more) field tests might be considered to reduce false positives and false negatives. In some cases, of course, only one test may be available; as long as it is properly validated or defined, having only one test should not prohibit its use.

Recommendation: Adopt proficiency and validation testing to estimate the false-positive rate and the false-negative rate and to ensure they are as minimal as possible.

MULTI-TIERED LABORATORY SYSTEM

Another way that the quality of laboratory results can be promoted is by matching complexity of tests to laboratory capabilities. Not all testing needs to or should be done in local laboratories. For example, local laboratories do not need to generate detailed strain

identification, which can be concentrated in a few specialized and accredited laboratories. If a local laboratory obtains a suspicious finding, testing can proceed up the system for the next and more technically challenging tests.

At the present time, a crime laboratory hierarchy is nonexistent. When setting up a multi-tiered laboratory structure for microbial forensics of bioterrorism agents, the model established during creation of the Laboratory Response Network (LRN) could be followed:

Level A: Includes all hospital and commercial reference laboratories that are able to obtain presumptive identifications of most of suspected bioterrorism pathogens. Such isolates or samples are referred to Level B, C, or D laboratories for confirmation.

Level B: Comprises ~200 laboratories that have been judged competent to perform agent identification confirmatory testing.

Level C: Includes 20-30 laboratories that perform antimicrobial susceptibility testing and typing of isolates.

Level D: Limited to only two (2) laboratories that can do all of the above, plus genome sequencing.

A hierarchy for laboratories proficient in testing food samples is still under development. United States Department of Agriculture laboratories can do agriculture testing, but the system needs to be expanded to bioterrorism microbes. California has mobile response units, but not all states may be at this level. Not all laboratories will be proficient at testing all pathogens, and a single lab may be at one level for particular agent but at a higher or lower level for others.

EDUCATION, TRAINING, AND COMMUNICATION ISSUES

A formal national program in microbial forensics is a major enterprise and establishing the necessary support systems and additional data will require considerable changes in knowledge, training, and attitudes. Education and re-training of professionals for this emerging field will be required. In addition, professionals and the public will need to become conversant with the new field.

PROFESSIONAL EDUCATION

As a first requirement, we need a corps of certified forensic microbiologists. To build the necessary capability in microbial forensics, practicing microbiology laboratory personnel should be trained in forensics, in addition to forensics personnel being trained to do microbiology. Forensic microbiologists need specialized training in the pre-analytic, analytic, and post-analytic phases of forensic testing. Microbial forensics training should be provided to enough personnel to provide full-time laboratory coverage. Microbial forensics training could be offered at local universities or perhaps at workshops at the American Society for Microbiology (ASM) General Meeting. Skills could be fostered by specialized fellowships in microbial forensics, with internships that would allow microbial academic researchers to work in a forensic setting, or vice-versa.

Forensic training should also be provided for staff persons in public health laboratories to capitalize upon existing infrastructure.

Training of first responders is a high priority. First responders may be Hazmat (Hazardous Materials) personnel, if a potential exposure is recognized before there are casualties, or emergency medical technicians, in the case of recognized casualties. In most cases, first responders will not be trained in knowing how to deal with a potential biocrime scene. Education is key to inform likely first responders how to determine whether there might be a biological threat. They must understand biological safety (biosafety) procedures and crime scene investigative procedures, as well as recognize the need to defer sample collection from a potential crime scene to authorized representatives from the local, regional, state, or federal public health or law enforcement officials. First responders should be trained in biosafety procedures by professionals in public health and/or law enforcement in the community or who are hired contractors.

For continuing education, certain conferences, such as the American Academy of Forensic Sciences, the American Society for Microbiology, the Cambridge Health Technology Institute, American Academy of Forensic Sciences, and Promega Human Identification meetings would be appropriate venues for sessions providing information of importance to forensic scientists, microbiologists, and law enforcement officials.

To facilitate communication among those working in microbial forensics, it could be helpful to establish a new journal or new sections in pre-existing journals on microbial forensics/biothreat response. Another possibly helpful step could be to organize a review issue of Microbiology and Molecular Biology Reviews (MMBR) on forensics issues or an ASM Press book with solicited reviews.

Interagency or intersociety collaborations could be fruitful ways to stimulate advances in this burgeoning

discipline. Three possibilities are collaboration between practitioners of microbial ecology and the National Aeronautics and Space Administration; collaboration among the National Institutes of Health (NIH), the Department of Energy (DOE), and the Environmental Protection Administration (EPA); and government/industry/academic fellowships and internships, possibly included in training grants. Joint research programs between the National Institute of Justice and the National Science Foundation (NSF) could also be productive.

Recommendation: Consider a new journal or new sections in existing journals on microbial forensics/biothreat response. Other possibilities include a review issue of MMBR on forensics issues or an ASM press trade book.

PUBLIC EDUCATION

To have a society that is supportive of the efforts that will be necessary for a successful microbial forensics initiative, considerable public education will be needed. An understanding about potential bioterrorism agents, preventive measures, manifestations of biocrime pathogens, and how they are detected and contained will be important to avoid panic when a bioterrorist strikes. Educating people about agents that cause syndromes similar to those caused by bioterrorism agents will also be a fruitful step and can avoid panic over natural disease outbreaks and hoaxes. Better education of the public about what different diagnostic approaches detect and how results of such tests are interpreted is needed. In particular, a broader comprehension of the meaning of false-positive and false-negative results would be an important advance. This is especially true if one decides to tolerate possible false positives in exchange for rapid evaluation. It is important that the American Society for Microbiology play an active role in passing on such material to the public.

Reaching the general public effectively with this type of information is difficult. Recommended avenues to pursue include:

- Education conveyed by local news media via public service announcements, with messages spanning different levels of sophistication.

- Radio spots for the general population on popular radio stations.

- Collaborations among ASM, the American Society of Virology, the Centers for Disease Control and Prevention (CDC), and microbiologists with other scientists, e.g., veterinarians, and other agencies to provide the best public information.

- Expanding a web site that is now available for exchange of information.

Locally, many different microbiology professionals interact with the media and public. We recommend ASM be involved in education of microbiologists on how to communicate better to the media and directly to the public. This could be done through ASM branch meetings or workshops at the ASM General Meeting.

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