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MINIREVIEW

Quality Sample Collection, Handling, and Preservation for an Effective Microbial Forensics Program

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Science can be part of an effective investigative response to a bioterrorism event or a biocrime by providing capabilities to analyze biological and associated signatures in collected evidence. Microbial forensics, a discipline comprised of several scientific fields, is dedicated to the analysis of evidence from such criminal acts to help determine the responsible party and to exonerate the innocent (6). A partnership among a number of government agencies, academia, and the private sector has been formed to better respond to and deter potential perpetrators of bioterrorism or biocrimes. This partnership leverages our national scientific and analytical capabilities to support activities of law enforcement agencies.

The Department of Homeland Security (DHS), whose mission is, in part, to respond to and to prevent acts of terrorism against the United States, has established the National Biforensics Analysis Center (NBFAC) (4, 6). The NBFAC, in partnership with the Federal Bureau of Investigation (FBI), (i) provides a state-of-the-art central laboratory for analysis of microbial forensic evidence and (ii) serves as a nexus for integrating the national resources to increase the effectiveness of law enforcement in obtaining the highest level of attribution possible in criminal cases where the weapon is a biological agent.

One approach used by the NBFAC to establish a sound foundation, to foster communication, and to facilitate integration across government and other agencies is to promote independent meetings, which address specific needs and provide a forum for input from the broader scientific community, on the best scientific practices in microbial forensics (5). As part of this ongoing effort, a series of meetings sponsored by DHS were held at the Banbury Center of the Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, to address specific issues for the enhancement of microbial forensic capability. One such meeting, held on 16 to 19 October 2005, focused on the collection, handling, and storage of samples. These issues had been identified at previous meetings (5, 6) as some of the most critical issues confronting a crime scene investigation and subsequent analysis of evidence. The participants represented diverse scientific entities within academia, the private sector, the national laboratories, and several federal agencies (Central Intelligence Agency, Centers for Disease Control and Prevention, DHS, FBI, Food and Drug Administration, and U.S. Department of Agriculture), some of which have been involved in evidence collection for purposes related to forensics, public health, or plant and animal health.

The collection and preservation of microbial forensic evidence are paramount to efficient and successful investigation and attribution. If evidence (when available) is not collected, degrades, or is contaminated during collection, handling, transport, or storage, the downstream characterization and attribution analyses may be compromised. Retrieving sufficient quantities and maintaining the integrity of the evidence increase the chances of being able to characterize the material to obtain the highest level of attribution possible. This paper presents issues related to the practices of sample collection, handling, transportation, and storage and includes recommendations for future directions for the field of microbial forensics and people

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participating in it. The recommendations apply to the NBFAC, as well as to other facilities and practitioners.

OPERATIONAL PLANNING

A terrorist event involving a biological agent can be either overt or covert. When it is overt, law enforcement (i.e., the FBI, which has the lead investigative role by authority) controls the investigation and works closely with public health authorities as appropriate. If it is covert, law enforcement may not be involved immediately. In some cases, the lag time for law enforcement involvement may be substantial. As is true for any crime investigation, this lag time could hamper opportunities to collect meaningful evidence. In either situation, there are many plausible crime scene investigative scenarios, ranging from searching a clandestine laboratory to identifying and containing a canister found in a subway.

The response to a biocrime or bioterrorist event can be divided into the activities carried out by first responders and the activities performed by hazardous material crime scene investigators. First responders may include health care workers, firefighters, police, paramedics, hazardous material personnel, veterinarians, or even concerned citizens. In a covert attack, some of the evidence, such as medical histories, clinical samples, and tissue samples collected by an attending physician, a medical examiner, or coroner, is likely to be collected prior to law enforcement involvement (33). Crime scene investigators usually follow first responders or a surveillance team that has gathered some preliminary information about the event. Thus, investigators who arrive to collect forensic evidence likely already have some prior knowledge about the crime scene. This knowledge should be used during the preoperational planning phase of a crime scene investigation, but it should not be used alone. Planning is requisite. For most bioterrorism cases, a detailed operational plan describing a strategy for the initial sampling, collection, and transport of evidence should be developed. The plan usually involves a focused search and collection process based on reasonable assumptions about the event. Preparatory work is essential because of the hazardous, chaotic, or even life-threatening environment presented to crime scene investigators. Investigators may have to wear personal protective equipment (PPE) (1) that limits mobility, the time available to work at the scene, and flexibility. Thus, crime scene investigators should plan, organize, and anticipate practices that facilitate the collection and handling of microbial forensic evidence on a timely and sometimes time-constrained basis.

The goal of a hazardous material crime scene investigator is to obtain sufficient biological agent and associated materials, when available, to support a meaningful analytical investigation for species and strain (and substrain) identification or for toxin identification. The biological agent itself, however, is not the only forensic evidence to consider. Related chemical and physical signatures, including by-products, and traditional forensic evidence, such as fingerprints, computer records, and trace evidence, can provide clues to the identity of the individual(s) who committed the crime. Where to sample, the method of sampling, and how many samples to collect are important considerations to obtain the right type, quantity, and quality of data to support attribution. Furthermore, it is essential to maintain the integrity of the evidence, to the extent possible, from the time of collection and during subsequent storage; otherwise, crucial and reliable forensic information can be lost.

Traditional basic crime scene investigation strategies apply to cases of bioterrorism or biocrimes (39). The first priorities are the care of victims (33) and the potential public health risks. The next priority is securing the crime scene. The event or suspected event may involve more than one scene, and determination of a scene and its extent may be difficult. Then coordination discussions should be held, in which law enforcement, public (or agricultural) health, and Environmental Protection Agency officials discuss their roles and capabilities in dealing with a biological attack, whether it is overt or covert. These discussions should include the best methods for the collection and handling of evidence, collection of specimens to assess the level of contamination in order to guide public health (or agricultural) investigations and restoration and recovery activities, and the proper PPE to use. Restoration and recovery activities to mitigate public health concerns (e.g., decontamination of facilities) should not occur until forensic evidence is collected for law enforcement purposes.

SAMPLE COLLECTION

The inherent rigidity in a standard operating protocol (SOP) for crime scene processing could be unwieldy and impractical in a bioterrorism event or biocrime, possibly even compromising evidence collection in some cases. Therefore, consultation among the different entities involved in a response should provide best-practice options framed on established guidelines (45). The plan can be modified after greater scrutiny of the crime scene or as more information is obtained during the course of the investigation.

The participants emphasized that it is not possible or even desirable to prepare a single, defined SOP (or even a number of them) to process the many possible crime scenes that may be encountered. However, lack of a specific SOP should not preclude attempts to collect critical evidence. If the forensic situation fits a pattern or template for which a sample collection methodology and/or sampling strategy already has been validated, then the sampling activities could be well-defined and more focused. However, in most microbial forensic cases, the sampling area, location, type of agent, substrates, and combinations of these variables are almost always novel. This may be particularly true for trace evidence. Indeed, some flexibility in the sampling processes must be allowed, because every crime scene is different and the variability of the presentation of microbiological evidence can be immense. Moreover, the sampling requirements for microbes or DNA evidence are likely to be completely different than those for different toxins.

Perhaps the best and most widely accepted approach for development of a sampling strategy in a particular case is to follow established guiding principles (for crime scene investigations) in combination with expert knowledge, including microbiological and biochemical knowledge, investigative experience, and common sense. Even without a prescribed sampling tool or methodology, sometimes innovation or intuitive means may be necessary as it is preferable to attempt to collect evidence rather than to ignore the evidence altogether. The operational sample collection strategy should accommodate the

uncertainty about the evidence being collected. However, when a novel or modified approach is used, all steps and the information accrued must be well documented for future reference and scrutiny.

The agencies involved in the investigation of buildings potentially contaminated with anthrax spores via the mail (38) determined that the best chance of recovering evidence for forensic analysis (as well as for determining an initial public health risk) was to focus on areas likely to be exposed to spores (36). A targeted sampling strategy is the appropriate approach and should be considered first when a biological agent is suspected or when information on the source of a potential biological agent is available. The targeted approach should not be confused with remediation sampling strategies, which are different than the sampling strategies used for crime scene investigations (see the discussion about the Government Accountability Office [GAO] report below). A defined approach allows maximum success in retrieving evidence, especially when the conditions for collection are hazardous or time constrained, as well as utilization of limited resources as effectively as possible.

Evidence may be collected by three general approaches. One approach is to collect the whole item and transport it to a properly dedicated biocontainment facility, previously shown to be free of contaminating biothreat agent signatures, for further sampling and analysis. This approach minimizes the time required for evidence collection, which is important when investigators are wearing PPE. Makeshift certified facilities may be designated for very large pieces of evidence, and the NBFAC facility may be used to accommodate smaller pieces of evidence. Once evidence is transferred to a secure location, more vigorous evaluation and detailed sampling can be conducted in a controlled environment under appropriate biocontainment conditions. However, some items may be too large or too bulky to be removed from the crime scene, the items may not be accommodated in a controlled laboratory setting, or there may be some concern about loss of trace evidence during transport. When the whole item cannot be recovered, an investigator can remove a portion of the item (this approach includes vacuuming, filtration, and/or water sample collection). Lastly, an approach that is particularly useful for collecting trace materials is swabbing or wiping materials or surfaces with appropriate sample collection devices.

Sample collection devices include dry swabs, premoistened swabs, wipes, high-efficiency particulate air vacuums and filters, and aspirating needles (7–9, 21, 31). Substantial experience exists within various government agencies, clinical microbiology, the Laboratory Response Network (19), and veterinary and agriculture practices (7, 12, 21, 35, 46) for guidance concerning the approach to use in a given situation. However, there are three major concerns regarding the use of sampling methods and collection devices. First, some of the methods and sampling devices have not been rigorously validated. It is not known which method and device yield the best collection efficiency or provide the best recovery of physical, chemical, and/or biological signatures. In some cases, sufficient material may not be available for sampling during an investigation even though adequate material may have been present at the crime scene. The assumptions made by crime scene investigators are more limited if the efficiency of the collection method is unknown. Second, a number of methods have been validated, but current security restrictions may hinder sharing the validation data between agencies. Thus, data on best practices, as well as data on practices that do not perform well, may exist but are not available to all workers who need them. Third, one should have a general understanding of the analyte or target signature that will be analyzed, and the collection method should be tailored appropriately. This is not an easy task for microbial forensics practitioners because of the uncertainty associated with each case, the diversity of bacteria, fungi, viruses, and toxins, and the stability of analytes, such as nucleic acids or proteins. What may be reasonable for collecting one microorganism might be deleterious for another (33). Also, what may solubilize one toxin may be ineffectual for recovering another toxin (35). Even differences in the background matrix carrying the evidence material may warrant changes in the sampling strategy. Thus, in some scenarios it might be good practice to collect multiple samples and use several different preservation modalities to accommodate different analytical schemes (see recommendations below for a strategy to begin to address the major concerns for sampling).

The methods and devices for evidence collection must be validated with regard to subsequent analytical processes. Consider a scenario in which a crime scene investigator uses a swab with a 15-cm diameter to collect evidence over large surface areas. Although validation testing shows that such a swab is effective for collecting microorganisms, most if not all diagnostic and analytical laboratories cannot accommodate such a large swab for sample processing (e.g., extraction of nucleic acids). This exaggerated example stresses the point that validation should be designed with consideration of the entire process from collection to analysis. Other examples to consider for DNA-based analyses are chemicals inherent in the swab material that copurify with DNA and/or environmental impurities such as metal ions or organic compounds that may preferentially bind to the swab during collection and may inhibit the PCR. Alternatively, swabs may contain soluble components that may be either cytotoxic for cell culture systems used to recover viruses or inhibitory for the growth of certain bacteria in culture.

Some materials designed to preserve a particular pathogen may impact negatively on the analytical assay. In the clinical laboratory, specimens containing bacteria tend to be delivered in general transport media (e.g., Amies or Stuarts) that contain some nutrients to maintain viability. Viral transport media usually consist of solutions such as phosphate-buffered sucrose or Hanks balanced salt solution with bovine serum albumin and some antibiotics to retard bacterial growth. Many commercial PCR or antigen detection kits provide proprietary transport media that have been designed to stabilize the analyte in question. There also are some general transport solutions for collection of samples for PCR analysis that lyse the bacteria or viruses and stabilize the nucleic acids (13, 18, 29).

The media generally used to collect and transport animal pathogens also were developed primarily to preserve proteins, nucleic acids, and pathogen viability. The stability of some animal viruses (e.g., foot-and-mouth disease virus) is markedly affected by pH and ionic strength. One of the most commonly used transport media is buffered tryptose broth. Buffered glycerin, internationally used to transport vesicular disease specimens, has been shown to preserve the causative virus at room

temperature for long periods of time. This medium is not used in the United States and has not been thoroughly tested for PCR inhibition. Glycerol, at different concentrations, may inactivate certain viruses while preserving others in clinical samples (25, 41, 42). A possible criterion for selecting an adequate collection and transport medium for viruses of agricultural concern could be the use of isotonic solutions with neutral pH (23). Additional guidance for collection of virus specimens can be found at http://www.who.int/csr/disease/avian_influenza/guidelines/humanspecimens/en/.

The number and intricacies of the areas under consideration emphasize the need for extensive training and knowledge to allow flexibility in the development of sampling strategies that are best suited for each set of circumstances.

PACKAGING, TRANSPORT, AND STORAGE

Obtaining an analytical result also can be affected by the manner and conditions under which a specimen is transported and stored. The same concerns discussed above with respect to sample collection also apply to packaging and transportation. Storage conditions differ for some microorganisms. For example, anaerobes die when they are exposed to ambient levels of oxygen during storage and therefore cannot be recovered upon anaerobic culture (28). The packaging or storage conditions required for a given microorganism also may differ depending on the sample matrix or physical condition (e.g., liquid versus powder). When there are copious quantities of microbial forensic evidence, some loss may be inconsequential, and various packaging and storage strategies can be applied. However, trace materials are very limited, and at times collection of only one sample may be possible. In such scenarios, efforts to maintain the integrity of the sample are more demanding and critical. The packaging, transportation, and storage conditions should be related to preserving the analyte or signature to be analyzed.

Clinical medicine has well-developed packaging, transport, and storage protocols (15, 16, 27, 37). Each specimen is transported in a package appropriate for the suspected microorganism and the type of specimen collected. Packaging for most routine pathogens and the pathogens on the select agent list (2) is defined, and the likely clinical specimens in which they reside are known (11). Commercial products are available for transporting clinical specimens. Transport packages designed for most bacteria are not adequate if a viral etiology is suspected. Specimens for culture should be transported to the laboratory as promptly as possible. Transport strategies that minimize damage, loss, contamination, or exposure to personnel are necessary (43). Some specimens can be transported at room temperature, and some should be transported on ice (see reference 44 for some recommended conditions). Most specimens should be stored refrigerated to maintain viability, preserve relative proportions, and minimize overgrowth of contaminants (blood is an important exception and should not be refrigerated). Many commercial clinical laboratories have developed efficient and effective methods for defining transport media and appropriate temperatures for particular classes of microbes. The volumes of samples prescribed for analysis allow for extra material, so evaluations can often be made when the sample is "improperly" transported. Under certain circumstances, the collection of a specimen for microbial forensics is more like the collection of a cerebrospinal fluid sample (14, 34) or a surgical biopsy from a patient where it may be difficult or impossible to obtain a second sample. Because it may be difficult to predict the optimal transport materials or process, a variety of options should be made available to crime scene investigators, and there should be consultation with experts, when possible, prior to packaging.

For food or plant materials, the recommended packaging and storage practices are similar to those used in clinical microbiology and have been well described (22). Sampling and sample plans for foods are discussed in detail elsewhere (17). Such methods depend on the nature of the food (e.g., whether it is a solid, semisolid, or liquid; the storage conditions [i.e., whether it is frozen, refrigerated, or at ambient temperature], and the type of packaging, if any, ensuring integrity of the food product). To minimize amplification during transport or storage, when possible, the sample should be maintained dry, frozen, or at least chilled. However, some microorganisms may be harmed if they are frozen. Buffered glycerol has been used to minimize injury due to freezing and thawing (24). Methods that do not cause significant decreases in the viabilities of specific organisms improve typing success (30).

If an act of bioterrorism were perpetrated against agricultural targets, sampling of crops and environmental materials would be required. Samples of plant tissues or associated materials, such as insects, nematodes, soil, or water, should be collected directly into a container with minimal contact. In the field, plant tissues are usually placed dry into paper or plastic bags and stored on ice until they can be refrigerated or processed. In some cases, adding a small amount of sterile glycerol to the plastic bag may delay tissue desiccation and preserve pathogen signatures. Plastic sandwich box humidity chambers can maintain humidity without directly wetting plant tissues or insects. Masses of fungal hyphae or spores and bacterial ooze have been collected from plant surfaces or soil into sealable containers containing sterile water, a mild phosphate buffer (3), or 70% ethanol depending on the type of analysis to be performed. Some microorganisms (e.g., bacterial ring rot, which can survive for 2 to 5 years in dried slime on equipment, burlap sacks, etc.) can survive without stringent storage conditions (32). Long-term storage of forensic microbial samples prior to analysis is often necessary; however, long-term storage conditions have not been well defined. Optimal storage conditions (freezing versus refrigeration or lyophilization, humidity, storage media) and sample longevities have been determined for only a few plant pathogens.

While some of the practices described above apply in general to collection of forensic samples, many times samples are collected from the environment surrounding a crime scene. In such cases, the state of the agent should be considered. For example, cooling of dry material may produce condensation that could alter its physical state and possibly interfere with subsequent analysis. As a general practice, collected samples are placed into sterile containers using dedicated sterile collection tools. The samples are then placed into prelabeled translucent ziplock bags for secondary containment and maintained at ambient temperature. A ziplock bag used as either primary or secondary containment for contaminated physical evidence may be contaminated during sample collection.

Therefore, the exterior of sample containers should be decontaminated. The protocols for packaging and transporting samples to laboratories for analysis are based on federal regulations designed to avoid inadvertent release of infectious substances (12). However, for forensic analyses, maintaining the integrity and authenticity of samples from the point of collection is paramount, and following minimal transport regulations may not be adequate. There is a need to validate transport and storage methods for as many agents and analytes of concern as possible. In addition, because remaining evidence should be made available to the defense for retesting, if desired, proper long-term storage conditions have to be validated.

GOVERNMENT ACCOUNTABILITY OFFICE REPORT

It is important to draw distinctions between investigations designed to gather microbial forensic evidence and investigations designed to determine whether a pathogen is still present, perhaps after a remediation effort. A recent GAO report provided recommendations concerned predominantly with remediation. However, some important areas of overlap with evidence collection for law enforcement purposes are instructive. The GAO report (40) addressed and was consistent with many of the issues discussed at the recent conference. The scientists in attendance generally agreed with and supported the findings of the GAO report in that validation of methods and processes was deemed necessary to achieve best practices. However, the conference attendees cautioned that the sampling strategy advocated by the authors of the GAO report for postremediation analysis should not be extrapolated to, nor is it the best approach for, microbial forensic or initial public health and agricultural health investigations.

The GAO report was critical of the targeted sampling strategy used to detect *Bacillus anthracis* in postal facilities in 2001. In this instance, government agencies collected samples where, in their best judgment, *B. anthracis* spores would most likely be found. The GAO report suggested that the use of a random and/or probability sampling strategy would allow determination of a level of statistical confidence for the absence of building contamination in cases when all sampling results are negative. As noted above, the report apparently pertains to remediation and the decision of when to consider a building safe for reoccupation. Whereas such verification sampling may be necessary to confirm decontamination of a building (10), it should not be confused with the different requirements of microbial forensics.

FORENSIC SAMPLING STRATEGY

In clinical and agricultural diagnoses, sampling is prioritized based on location, the type and extent of symptoms, prior knowledge based on sound principles, and the materials available rather than randomly across the body of an infected individual, a field, or any location. Likewise, it would not be productive for most forensic or clinical investigations to use a randomized sample collection strategy. For example, if a patient presents with fever, difficulty with breathing, and a productive cough but no headache or stiff neck, a physician would likely obtain a sputum sample to search for a possible microbial etiology rather than randomly gather samples that include a lumbar puncture for cerebrospinal fluid or collect gastrointestinal samples to look for parasites. Similarly, fever, headache, and a stiff neck would direct the physician to consider meningitis as the diagnosis and target sampling to the cerebrospinal fluid to determine if an infection was present. For investigations involving plants, if necrotic lesions are present, it is best to obtain samples from the lesion edges, where living plant tissue supports active pathogen growth. Certain specialized pathogen structures, such as the galls of smut fungi or the tumors produced by the crown gall bacterium, may be collected directly. In a food poisoning case, consumption of a food may be epidemiologically associated with illness resulting from contamination with a food-borne pathogen. To identify the microorganism, it would be more effective to collect samples from the individuals who became ill than to collect samples from randomly selected individuals in the population. In one example of a food-borne outbreak that involved a targeted investigation (and subsequent reexamination), strawberries were associated initially with development of illness in a number of people. Although the cause was subsequently shown to be *Cyclospora cayetanensis*, a gastrointestinal agent found in raspberries from Guatemala (20), targeted sampling was clearly advantageous in collecting the most informative samples. Similarly, the presence of castor beans or bean mash at a crime scene, or even access to internet records, would suggest the use of directed sampling methods for the collection of suspected ricin. Just as a clinical investigation or a food-borne illness investigation should use the patient history, physical examination, and blood work to direct sampling, a forensic investigation should use available information to guide sampling. In other words, procedures that would have the highest diagnostic yield are given priority.

Certain factors can diminish the likelihood of a positive finding with samples. Using food-borne contamination as an example, these factors include increased time from contamination to sampling, uneven dispersal of the pathogen in the contaminated food, a very large volume of food to be sampled, large batch size, short shelf life of the food, rapid turnaround of implicated foods, and incorrect linkage between the food and illness. These factors suggest that a sampling regimen targeted to only the implicated food(s), its most probable source(s), or the site(s) of origin for similar foods is the preferred sampling approach.

Despite the clear utility of targeted sampling in forensic (and epidemiologic) investigations, statistically derived or random sampling strategies may be useful sometimes. Although statistically derived or random sampling strategies have not been used routinely in the forensic sciences, it may be necessary in some instances to understand sample-to-sample variability when workers try to compare other factors across the environment. An application to forensics would be collecting background samples for determination of endemicity, when large areas or quantities (e.g., shipments of a particular crop) need to be sampled to look for a possible contaminant, or for surveillance. In this respect, biogeographic considerations of microbial pathogens also are important; the theories and empirical observations regarding biogeography may provide important clues as to the endemicity or introduction of the specific pathogen identified during the investigation (26).

The sampling strategy employed should take into account the a priori knowledge about the crime scene, the science available for characterizing the pathogen being sampled, the different effects on recovery, the different surfaces that may require specific sampling regimens, the microorganisms sampled that might be viable but noncultivatable under the conditions used for sampling, the possibility of different physiological states of an organism (e.g., biofilm formation), and other signatures (e.g., additives, stabilizers, morphology, and isotopes) that may be forensically informative. If a bioterrorism event is suspected to have occurred within a building, several scenarios may be plausible. The release point may be easily traced to a specific container or visibly contaminated small area. Alternatively, the exact location of the release may be unknown, but there may be evidence (e.g., victims and disturbed areas) that points to a general location. There may also be cases requiring a comprehensive systematic sampling strategy where the presence or absence of the agent or material may be of investigative value. Finally, there may be little evidence to indicate where the release, if any, may have occurred. Under the first two scenarios, one would want to perform targeted sampling near release points and on surfaces near the release points where it is most probable that the biological agent may have been deposited. Only in the last scenario might some probabilistic sampling be prudent. Nevertheless, the initial sampling may still begin, for example, with the ventilation system filters to determine if there was a release. Development of a knowledge and information database would facilitate the formulation of sampling strategies for different situations.

RECOMMENDATIONS

One of the goals of the DHS-sponsored Banbury meetings is to identify the most pressing needs in microbial forensics so that law enforcement, the NBFAC, and other scientific community assets can focus their efforts on closing identified capability gaps. For enhancing the tools available for sample collection, handling, transport, and storage and for obtaining maximal effectiveness in the application of investigative methods, the following recommendations are offered.

(i) Existing collection, storage, and transport protocols should be housed and curated at a single site. Such a database would allow a preliminary comparison of methods to determine which methods are sufficiently validated, what further validation may be necessary, which methods are likely to succeed, and which methods are not effective. This would reduce duplication of effort and allow scientists to build on previous knowledge to improve processing and analytical methodologies. Moreover, an available knowledge database would facilitate development of the best operational plans. All current methods, including those used by all federal agencies and those described in the literature, should be placed into the database, which should be accessible to appropriate entities. In addition to the protocols, the data should include information on the sample collection device, the type of agent (e.g., enveloped virus, RNA virus, toxin, bacterium) or other forensically related material, what downstream analytical methods have been used on the collected or recovered material (e.g., extraction methods, live agent culture, PCR-based assay, elemental analysis), the storage conditions (e.g., room temperature, 4°C, frozen at −20°C, −70°C), the transport conditions and media, the interpretation guidelines, the long-term storage conditions, and supporting validation data.

(ii) Protocols need to be validated with a broad spectrum of bacterial species or strains, viruses, and toxins. There are many protocols that have not been rigorously validated, and this lack of evaluation limits the capabilities of crime scene investigators to make the most effective decisions when they develop operational plans. In addition, subsequent interpretation of analytical results could be compromised without validation data. Discipline-wide validation criteria should be developed. While it is not possible to prescribe every possible tool or method used to survey, capture, swab, assay, or otherwise detect pathogens that may be found at a crime scene, some general criteria are needed to guide researchers and developers. These criteria should include sensitivity, specificity, recovery efficiency, maintenance of integrity, impact on analytical assays, and baseline disease and pathogen data.

(iii) Guidelines for collection of evidence at bioterrorism and biocrime scenes should be established. The best approach for searching a crime scene is the use of targeted strategies grounded with established guidelines and experience. Certain law enforcement personnel are trained specifically in the processes of crime scene investigation. The guidelines used for collecting and handling traditional forensic evidence should form the baseline for microbial forensic investigations. Indeed, the protocols developed by the Hazardous Material Response Unit at the FBI are based on such practices. Therefore, the crime scene investigation protocols of the FBI's Hazardous Material Response Unit should become the initial de facto national guidelines. The principles and guidelines should be made available to other appropriate organizations, so that they can benefit from previous experience; also, this could facilitate comparisons of information collected by different agencies. In addition, availability fosters peer review and leads to rigorous evaluation and thus could improve the current guidelines. The ultimate success of recovering evidence during a crime scene investigation is dependent on an understanding of the type(s) of organisms (fungi, bacteria, viruses, or other parasites) or other organic and nonorganic materials that might be present. Knowledge of the most effective tools for collection and recovery and of the best storage conditions is essential for improving the formulation of an operational plan, facilitating identification of the types of effective signatures, and minimizing the impact of potential inhibitors. Empirical data on validation studies should also exemplify scenarios to illustrate why and how collection tools and methods have to differ depending on the downstream methods used for detection and pathogen identification. Knowledge of what public health workers have already developed with respect to outbreak investigations would also be helpful for investigations, particularly those involving food.

(iv) Finally, there is a need for training (12). Hazardous material response crime scene investigators responding to known crime scenes are likely trained in proper practices. However, there will be cases where law enforcement or medical examiners may be responding to a more traditional crime and unknowingly enter a scene where a biological agent has been involved or is being prepared. These investigators may not be aware that the scene is hazardous or that there are

signatures indicative of bioweapon preparation or production. Necessary training should be made available so investigators are more cognizant of the indications of the presence of suspected microbial biohazards (e.g., sophisticated equipment, egg incubators, yogurt makers, beer-brewing equipment, bleach, pressure cooker, petri dishes, flasks, pH paper, improvised fermenters, mills, PPEs, sprayers).

First responders at a crime scene are likely to be local personnel or public health personnel and not necessarily individuals affiliated directly with law enforcement. In most cases, their top priorities are the health of victims, limiting disease spread and damage, and public security rather than preservation of a potential crime scene and evidence collection. Making these first responders cognizant, or better yet appropriately training them, would be beneficial to maintaining operator safety and preserving evidence that may otherwise be lost.

CONCLUSION

The purpose of the October 2005 DHS Banbury meeting was to identify gaps and make recommendations regarding sample collection, handling, and preservation of microbial forensic evidence. We report the nature of these discussions to inform the greater scientific community of ongoing directions in the field of microbial forensics. The microbial forensic investigation, its success, and its impact are dependent upon the initial phases of a crime scene investigation, which rely heavily on the collection, handling, and preservation of physical evidence. If these procedures are not developed as well as possible, the entire process is weakened. We stress that efforts should be intensified in the areas described here to ensure capability for robust development, validation, use, and reliability of microbial forensics to support the attribution of crimes involving the use of biological weapons. Additional or alternate viewpoints intended to bolster these research and development plans and, most importantly, to contribute to the closing of gaps that can enhance capabilities of achieving attribution in acts of bioterrorism or crimes are welcome.

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