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The National Bioforensic Analysis Center

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History of the NBFAC

The anthrax letters of 2001 prompted the realization within the US Government that forensic analysis of evidence from a biological terror event or biocrime was not something that could be easily performed in conventional laboratories. Forensic laboratories could not handle the evidence from such an event safely because the Federal Bureau of Investigation (FBI) laboratory and others similar to it are not typically built with biocontainment capabilities, and so evidence contaminated by a biological threat agent such as *Bacillus anthracis* puts the forensic laboratory staff at risk. Conversely, biocontainment labs (that is, those with BSL-3/BSL-4 operations that can safely contain threat agents) are normally used for research and/or countermeasure development purposes and are rarely equipped and maintained with the needs of a forensic investigation in mind (strict workflow and cross-contamination control measures, for example). The combination of biocontainment and forensic capabilities simply did not exist in the United States in 2001, and so during the early course of the FBI's "Amerithrax" investigation, local and state public health laboratories, the Centers for Disease Control and Prevention (CDC), military biodefense laboratories including the US Army Research Institute of Infectious Diseases (USAMRIID), the Naval Medical Research Center (NMRC), and the Armed Forces Institute of Pathology (AFIP), and university laboratories were used for containment laboratory, bacteriological, molecular biology, and electron microscopy support. Although these laboratories contributed significantly to the rapid identification of the *B. anthracis* Ames strain in all the mailed letters, they were largely public health or research laboratories and did not have established procedures for the processing of environmental forensic samples, the ability to support traditional forensic techniques such as fingerprint or trace fiber analysis within biocontainment, or the ability to handle large and bulky evidentiary items such as mailboxes or car seats. In addition, they did not have procedures in place to strictly control for the potential of nucleic acid or antigen cross-contamination in their laboratories, and they had unique public health, biodefense, and research missions, which were significantly

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interrupted and were not available for the duration of a long investigation. Thus, it became very clear that a new biocontainment laboratory devoted to a forensic mission was needed.

In direct response to this need, Homeland Security Presidential Directive-10 (HSPD-10) (Bush, 2004) was drafted and states that "We have created and designated the National Bioforensic Analysis Center of the National Biodefense Analysis and Countermeasures Center, under the Department of Homeland Security, as the lead Federal facility to conduct and facilitate the technical forensic analysis and interpretation of materials recovered following a biological attack in support of the appropriate lead Federal agency." More recently, the National Biodefense Strategy of 2018 (Whitehouse, 2018) reaffirmed the need to have a forensic capability to support investigation following biological events to "Conduct operations and investigations, and use all available tools to hold perpetrators accountable." The National Bioforensic Analysis Center (NBFAC) provides dedicated staff, containment laboratories, equipment, and procedures to conduct operational forensic analysis to support the development of scientific data that can be used by investigators for attribution analysis of planned and actual biocrime and bioterrorism events. The NBFAC opened its doors with CDC-certified and newly renovated and equipped BSL-2 and BSL-3 laboratories within USAMRIID in May 2004, and within hours it received its first samples in support of the ongoing FBI Amerithrax investigation. In 2010, the NBFAC moved its laboratories into the new NBACC building on the National Interagency Biodefense Campus at Fort Detrick, Maryland, and has been operating in that space since that time.

NBFAC operations

NBFAC's concept of operations was designed with three main goals in mind: first, to apply the best possible scientific approaches to the analysis of evidence in support of biocrime/bioterror investigations; second, to provide the nation with a facility in which those analyses as well as traditional forensics could be performed safely and securely on evidence that may be contaminated with the most dangerous pathogens in the world; and third, to provide an environment in which those analyses could be done with the highest confidence and meet the standards of the forensic and legal communities. Each of these significant challenges, which require continual effort to maintain, had not been fully tackled before the establishment of NBFAC.

Scientifically, NBFAC is composed of a number of independently staffed groups, each composed of a mix of PhD, MS, and BS-level scientists who are specialists in their respective area. These groups represent the traditional disciplines normally found in a biocontainment laboratory—bacteriology, virology, toxinology, molecular biology—as well as the functional areas that support method-based approaches such as genomics, bioinformatics, analytical chemistry/mass spectrometry, and electron microscopy. Each group has its own dedicated labs for both casework and capability expansion (i.e., development, evaluation, and validation of new methods) so that new methods can be tested without the possibility of contaminating spaces or equipment that is being used for casework. There is also a separate group of sample processing specialists whose role is to manage evidence handling and storage and facilitate the transfer of evidence from investigators to groups for analysis.

Each of these groups maintains capabilities at BSL-2, BSL-3, and BSL-4. In addition, NBFAC maintains space at BSL-3 and BSL-4 to support traditional forensics such as fingerprint analysis, document exams, and computer analysis on contaminated evidence within biocontainment. This eliminates the need for potentially damaging inactivation procedures that would otherwise be needed to safely remove the

evidence to a conventional forensic laboratory. All of this is done to ensure that NBFAC can support analysis of any biocrime-derived material, no matter what threat agent might be present. This range of biosafety levels requires a great deal of training as well as redundant equipment, but it makes it possible to conduct bioforensic analysis safely on any sample.

Perhaps the most unique aspect of NBFAC's design and operations is its adherence to the principles of forensic laboratories. Where many biocontainment laboratories are built with the assumption that BSL-3 and BSL-4 space are at a premium and thus must support multiple projects, NBFAC's spaces are typically used by a single group for a relatively small set of methods. Similarly, NBFAC's workflows are designed to be unidirectional so that samples move on a directed path through the labs as they are analyzed; this is counter to the flexibility that most biocontainment laboratories build into their design. Finally, NBFAC spends a significant portion of its resources every year gaining and maintaining ISO 17025 accreditation for its methods. This accreditation provides third-party oversight of its methods and processes and facilitates the acceptance of NBFAC's analysis during legal review (e.g., the Daubert standard), but it requires significant effort from NBFAC's staff and also means that spaces and equipment are often "locked" into being used for a single purpose.

NBFAC science

One of the biggest challenges NBFAC has is that its core mission is constantly evolving and so must its science. The emergence of new infectious agents such as Middle East respiratory syndrome coronavirus (MERS-CoV), as well as variants of well-known microbes such as Ebola virus, means that the list of threats is always growing, and NBFAC's methods must keep up with the pace. In addition, even relatively well-characterized agents such as *B. anthracis* have turned out to be much more complex than previously thought, as large-scale genome sequencing and phenotypic surveys have identified very close relatives that are harmless as well as more distant relatives that cause anthrax-like disease, and again NBFAC's analytical tools must be adjusted to eliminate both false positives and false negatives. The lesson here has simply been that NBFAC must maintain a broad repertoire of orthogonal analytical methods so that multiple types of analysis can be brought to bear on a given sample and also that NBFAC must establish a culture of continual improvement so that the methods can evolve with a changing threat landscape as well as rapidly advancing technology.

The general scientific approach that NBFAC has adopted uses a wide range of both agent-specific and agent-agnostic methods (that is, method-based rather than agent-based). Agent-specific methods include real-time polymerase chain reaction and immunological assays that allow for the extremely sensitive detection of high consequence microbial and toxin targets such as *B. anthracis*, *Yersinia pestis*, and ricin. Although NBFAC maintains a large repertoire of agent-based assays to cover a wide range of targets, it is readily apparent to anyone familiar with the world of biology that this approach will eventually fall short, simply because there are so many possible biological threats and no organization can validate and maintain assays specific to each. To meet this challenge and to extend NBFAC's analytical capability to newly emerging, engineered, or even synthetic biological agents, several different types of agent-agnostic approaches have been established. These include electron microscopy, broad bacteriological and virological culture, genomics, and mass spectrometry. Although the agent-based assays are often more sensitive, these method-based approaches allow for a much more flexible analysis where the question being asked is not simply "Is Agent X in the sample?" but also

"What is in the sample?" Together, agent-based and method-based approaches allow NBFAC to provide exquisite sensitivity in the detection of known biothreats, as well as to be ready for the threats that have not yet been seen. The combination of agent- and method-based approaches allows NBFAC to tailor its analysis to the needs of the specific investigation, whether the priority of that case is speed, sensitivity, specificity, or all of the above. The use of multiple complementary approaches also allows for confidence in reporting, whether the results are from methods that have been used for decades or from cutting-edge techniques that are much newer.

NBFAC's mandate to provide cutting-edge scientific support to the investigators means that whenever its scientists are not performing casework, they are working on developing and evaluating new methods. These efforts are particularly critical for the method-based approaches such as genomics and bioinformatics, where technological advances are occurring very rapidly. New sequencing platforms and new software tools often offer new capabilities and must be evaluated relative to NBFAC's mission and goals and then rapidly validated and integrated if they add value. The same is true of other areas such as analytical chemistry and mass spectrometry, and while agent-based assays are still also added as needed, the bulk of NBFAC's developmental work is focused on method-based approaches. Significantly, this work is published and/or made available as open-source material whenever possible—this allows the greater scientific community to work with NBFAC scientists on things like new software tools, and it provides another mechanism for external review of NBFAC's methods.

The last key piece of NBFAC's scientific strategy is its relationships with partner organizations. Early in NBFAC's history, it maintained close ties with many other laboratories that had expertise in specific areas such that despite the fact that it had relatively few capabilities itself, it could serve as the "hub" in coordinating the actions of many different "spoke" laboratories to conduct a complex analysis for investigators. Spoke laboratories included the FDA, several DOE National Laboratories, the Plum Island Animal Disease Center, and a few other academic and private laboratories. As NBFAC became more established, the expertise that these other laboratories provided were incorporated into NBFAC's in-house competencies, but the relationships were largely maintained, and many of NBFAC's capability expansion projects are still done as collaborations with outside organizations. The continued success of the NBFAC requires coordination with partners in academic and government laboratories. In the past 10 years, NBFAC scientists have published more than 60 peer-reviewed manuscripts describing the development, evaluation, and/or demonstration of new bioforensic methods, and well over half of them have involved collaborations with partners throughout the scientific community. These relationships have been absolutely critical to NBFAC's success in establishing itself as a leader in biodefense and in being able to offer investigators the best possible scientific support.

NBFAC and the future of bioforensics

Driven by both evolving threats and new technologies, NBFAC's mission and capabilities are expanding. Where the focus was once microbial forensics and pathogen detection, NBFAC is now fully embracing the concept of bioforensics—the analysis of any biological material that may be part of a biocrime/bioterror investigation. This concept aligns with the establishment of method-based approaches that are not only capable of detecting high-consequence biothreats but can also produce a profile of an entire sample, perhaps identifying background components that represent unique signatures, such as the *Bacillus subtilis* contaminant present

in some of the Amerithrax letters. In the future, NBFAC's strategy is to focus not on specific threats, but to aim to fully characterize the nucleic acids, proteins, and small metabolites in a bioforensic sample using a combination of genomics, proteomics, and metabolomics. This does not mean that the agent-specific assays currently in use will be discarded, as it will likely be years or decades before the method-based processes can match the single molecule sensitivity that the agent-based methods often provide. It simply means that NBFAC will continue to invest in method-based assay development, while using agent-based assays or combined approaches such as amplicon sequencing when sensitivity or high throughput is required. It also means that the computational methods needed to support genomic, proteomic, and metabolomic analysis will be a key element of NBFAC's development work going forward, as will the inferential and predictive methods that aid in interpreting those data and identifying new or hidden threats.

Key to this strategy will be continuing NBFAC's partnerships within broader scientific community because in many areas continued expansion of NBFAC's capabilities will require resources beyond what NBFAC can directly support. As an example, advances in genome sequencing have resulted in rapidly expanding sequence databases that offer improved resolution to genomic analysis, but require more and more computing resources. It has become clear in recent years that machine learning and artificial intelligence might offer more effective ways of processing these data. NBFAC has established collaborations with partners in the DOE National Laboratories as well as in academia that leverage other groups' expertise to produce advanced solutions in bioforensic analysis. NBFAC will continue to seek partnerships and expand capabilities that ensure readiness to rapidly identify any threat in a bioforensic sample.

Conclusion

The NBFAC has and will continue to have a significant impact in protecting the nation from biocrime and bioterrorism. As part of its long-term goals for the future, the NBFAC is working toward the ability to fully characterize a bioforensic sample using a combination of method-based approaches, and in doing so to be able to detect any biological threat in any sample. Through its continuously available dedicated laboratories and staff in addition to ongoing capability expansion to meet new, emerging, and potential unknown biological threats, the NBFAC stands ready now and in the future to rapidly support attribution investigations to successful closure.

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