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NATIONAL SCIENCE AND TECHNOLOGY COUNCIL

# National Research and Development Strategy

for Microbial Forensics

## **National Strategy to Support Research in Microbial Forensics Attribution Investigations and National Security**

### **Introduction**

As described in NSPD-33 and HSPD-10, the essential pillars of the National Biodefense Policy of the United States consist of: Threat Awareness, Prevention and Protection, Surveillance and Detection, and Response and Recovery. As part of the “Surveillance and Detection” pillar, “attribution” is specifically discussed with respect to biological attacks and can also be used pertaining to an investigation of a planned attack. Attribution is the investigative process by which the United States Government (USG) links the identity of a perpetrator or perpetrators of illicit activity and the pathway leading to criminal activity. Making a determination of attribution for a covertly planned or actual biological attack would be the culmination of a complex investigative process drawing on many different sources of information including technical forensic analysis of material evidence collected during the course of an investigation of a planned attack or material evidence

resulting from an attack. During the course of an attribution investigation these sources of information would generate many investigative leads and help draw connections between places, events and a possible pool of suspects <sup>1</sup>.

In addition to the traditional types of forensic evidence such as fingerprints, hair and fibers and human DNA, forensic material collected as part of a biological attribution investigation will yield unique types of microbiological evidence that are specific to the nature of the potential attack or the attack itself. Examples of such microbiological evidence could include; viable samples of the microbial agent, protein toxins, nucleic acids, clinical specimens from victims, laboratory equipment, dissemination devices and their contents, environmental samples, contaminated clothing, or trace evidence specific to the process that produced and/or weaponized <sup>2</sup> the biological agent.

A statistically sound scientific foundation supports the forensic capabilities used in traditional criminal investigations to generate investigative leads, determine inclusion and exclusion for questioned samples when compared to known references and establish identity. Current capabilities to forensically characterize microbiological evidence in support of an attribution investigation are limited primarily to detection and identification which, while important, only begin to scratch the surface in terms of forensic requirements for detailed characterization and comparative analyses. Microbial Forensics <sup>3</sup> is the emerging interdisciplinary field of microbiology devoted to the development, evaluation, validation, and application of methods to detect and fully characterize microbial samples containing a biological agent or its components for the purpose of statistically meaningful comparative analyses. These methods will provide data for attribution investigations involving pathogens, toxins or other biological

<sup>1</sup> 'Suspect' in this context can refer to an individual, group or hostile nation state.

<sup>2</sup> "Weaponized" agents are those altered to enhance effectiveness as a weapon.

<sup>3</sup> Microbial forensics has also been referred to as 'bioforensics' and 'forensic microbiology.'

materials that are collected as part of an investigation of a planned or actual biocrime or bioterrorism attack on people, animals or plants.

As a result of the Anthrax attacks in the fall of 2001, a considerable government investment was made in the scientific analysis of evidence resulting from the attacks. These investments did much to stimulate the field of microbial forensics and continue to

do so. However, microbial forensics is nonetheless still a nascent field facing broad and complex scientific challenges.

NSPD-33/HSPD-10 established the National Bioforensic Analysis Center (NBFAC) within the Department of Homeland Security's (DHS) National Biodefense Analysis and Countermeasure Center (NBACC). The NBFAC, an internationally accredited laboratory, is primarily operational in nature and is not focused solely on research and development. Advancing the field of microbial forensics to provide the NBFAC and other US Government organizations with robust capabilities to detect, identify and characterize biological agents will require a sustained investment in multidisciplinary basic research, focused technology development and robust method validation.

**Purpose:**

The purpose of the *National Strategy to Support Microbial Forensic Research* is to guide and focus the research efforts of the US Government to advance the discipline of microbial forensics and provide the nation with the most scientifically sound and statistically defensible capability to provide scientific data to support attribution investigations of a potential or actual biological attack. The *Strategy* has three primary goals:

- I. Develop a **strategic microbial forensics research agenda** that will produce a national microbial forensic capability that is ultimately capable of high confidence, robust detection, characterization and comparison of biological agents in forensic samples.
- II. Promote **interagency communication, coordination and information sharing** on microbial forensics research and development efforts.
- III. Develop effective interagency **education and training** on microbial forensics designed to inform policymakers and scientific and technical personnel.

This *Strategy* shall form the framework of an interagency implementation plan that will begin to address research and interagency communications issues that are required for an enduring national capability in microbial forensics.

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**Goal I. Develop a Strategic Microbial Forensics Research Agenda that will Produce an Enduring National Microbial Forensic Capability that Supports Sensitive Detection, Characterization and High Confidence Comparison of Biological Agents and or Their Components in Forensic Samples.**

A directed strategic microbial forensics research agenda that meets the requirements delineated by those government agencies conducting attribution investigations will guide investment to build a microbial forensic capability consisting of both genomic and non-genomic approaches for the forensic characterization and analysis of microbiological evidence supporting an attribution investigation of a potential or actual biocrime or bioterrorism attack. An effective microbial forensic capability must include reliable, rigorous and sensitive techniques to collect forensic samples, detect and identify forensically relevant “signatures”, and fully characterize forensic evidence. Additionally, a microbial forensic capability must be able to address the requirement to conduct comparative sample analyses in order to query known and questioned samples and draw inferences relating to the process used to produce a pure sample, the provenance of a sample or relatedness between samples. Rigorous quality standards must be applied at every level of the forensic process from sample collection, through sample analysis and data analysis to the reporting and the interpretation of results. In order to meet these challenges in microbial forensics, a number of research goals will have to be achieved. In some of these areas there is ongoing work while in other areas there is very little. This strategy serves to highlight important areas for research and provide recommendations for action in those areas based upon requirements of those conducting attribution investigations.

**Objectives:** Continue to expand national microbial forensic capabilities and develop new capabilities in the following areas: A.) sample collection, processing, preservation, and recovery and concentration of microbial pathogens and signatures from collected samples B.) sensitive signature detection and characterization C.) orthogonal methods for conducting forensic comparisons between samples to include the basic scientific research, which is required to build the foundational supporting data that will enable forensic comparisons to be made and interpreted, D.) validation of existing technologies for new application to microbial forensic problems, development of new technologies, and development of new bioinformatics analysis tools and creation of new theoretical frameworks for data analysis and interpretation.

### **A. Sample Collection, Processing, Preservation and Recovery and Concentration of Microbial Pathogens and their Signatures from Collected Samples for Microbial Forensic Analyses**

Actions:

1. Collect and evaluate all the work conducted across the US Government and academic sectors that has focused on the collection of microbial samples, preservation, recovery and concentration of microbial agents and their signatures from collected samples and use the results of the evaluation to identify current research gaps and consolidated research efforts to avoid duplication.
  
2. Develop and improve methods for the collection, processing, preservation and recovery of microbial agents and their signatures from microbial forensic samples that do not interfere with subsequent forensic analyses of the sample.
  - 2.1. Methods are needed to collect, process, and recover a wide range of human, animal, and agricultural microbial agents from a broad range of common surfaces, matrix types and sample collection devices.
  - 2.2. Methods are needed for the collection of trace microbial forensic evidence applicable to the recovery of viable organisms and non-viable trace signatures from a variety of sample collection environments.
  - 2.3. An interagency working group shall be identified or, if none exists, formed, to develop scientifically acceptable standards of performance and the path to validate the approaches.

### **B. Microbial Forensic Signature Development, Detection, and Characterization for Known, Emerging, Enhanced, Genetically Engineered Advanced and Synthetically Derived *de novo* Agents:**

Actions:

1. From among the total spectrum of human, animal, and agricultural pathogens, construct a prioritized list of microorganisms and toxins of biological origin that are assessed to be of high consequence in terms of actual threat or perceived existing vulnerability. Use the list to drive directed forensic assay development.
2. Develop sensitive and broad detection capabilities using both traditional microbiological culture, molecular (nucleic acid - based/ protein-based) and immunological detection and next generation technologies for the identification, characterization and comparison of all identified human and agricultural bio-threat pathogens and toxins including, new emerging microbial agents, enhanced microbial agents and advanced microbial agents to include a capability for *de novo* or synthetically derived microbial agents.

3. For additional levels of forensic characterization identify applicable phenotypic approaches such as, metabolomics, metabolic phenotype profiling techniques, serotyping, carbohydrate cell surface profiling, Membrane Fatty Acid Analysis (MIDI) and phage-typing that are useful across the range of human and agricultural bio-threat agents. Assess the limits of and utility those approaches as forensic tools.
4. Develop sensitive and specific forensic assays that can detect, identify and quantitate the amount of biologically active toxin of biological origin <sup>4</sup> present in a variety of commonly encountered environmental and food matrix types.
5. Develop sensitive and specific physical/chemical analytic capabilities that are capable of additional exploitation of evidentiary samples to identify and characterize preparation and process related signatures associated with microbial forensic samples.
6. Develop libraries of standard microbiological reference materials required, for the development and validation of microbial forensic methods, and for forensic comparisons to known isolates. Reference standards must be made available to assay developers and any independent assay validation group. Reference material must be well curated to insure the quality of the reference product used for comparative

analyses.

### **C. Forensic Sample Comparison and Generation of the Required Supporting Foundational Data.**

Actions:

1. Develop and execute a theoretical framework and set of forensic interpretation guidelines to define what is meant by a genetic “match” when comparing the genetic sequence of microbiological samples across the spectrum of microbial agents to include known agents, emerging agents, enhanced agents, advanced agents and *de novo* synthetic agents.
2. Engage experts in the fields of microbial ecology, epidemiology, and agriculture to help develop approaches to understand the spectrum of natural diversity and endemicity, of microbial agents that would be informative for forensic purposes and sample comparisons.
3. Conduct a survey of all currently available full genome sequences from microbial agents to include pathogens and near neighbor to assess if the number and diversity is

<sup>4</sup> The phrase Toxin of biological origin in this context refers to any toxic substance which is produced by a microorganism, plant, animal, insect or fungus that is dangerous to humans or agriculture and of biological weapons proliferation concern. Examples include but are not limited to ricin, botulinum toxin, and bioregulatory peptides.

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adequate for forensic purposes in terms of assay development, characterization, assay validation or interpretation of genetic comparisons. Based on the survey recommend additional strains for whole genome sequencing.

4. Develop and validate non-genetic orthogonal methods to conduct sample matching of microbial forensic samples from an investigation of a planned or actual biological crime or attack.
5. Continue to support a comprehensive research program on understanding genetic diversity in microbial populations and communities as it relates to both bacteria and viruses. Focus research on the forensics implications associated with bacterial and

viral population dynamics and environmental effects to genetic stability, gene transfer, mutation rates and other phenomena affecting the genome.

**D. Leverage of existing and next generation genomic and non genomic technologies for new application to microbial forensic problems, development of new technologies, and creation of bioinformatics analysis tools and new theoretical frameworks and models for data analysis and interpretation.**

Actions:

1. Continue to support the development of rapid and cost-effective high throughput sequencing and closure technologies that can be used to generate high confidence whole genome sequence data and genetic variation data for any known and unknown microorganism.
2. Continue to develop a specific bioinformatics genetic toolbox for microbial forensics, which addresses the unique requirements of forensic genetic comparisons.
3. Conduct a market survey of microarray and mass spectroscopy and other high throughput technologies that might be appropriate for forensic applications to identify the most promising candidate technologies, approaches, suppliers and types of systems (nucleic acid and protein-based). As part of the survey, address the a) maturity of the technology, b) experience of the producer, c) type of forensic application for which the platform is appropriate, d) cost and, e) possible lifespan of the technology, and f) benefit of the technology over traditional RT-PCR/Sanger methods.
4. Continue to support other genomic and non genomic high throughput technologies that can be applied to microbial forensic capabilities including DNA microarrays, proteomics, metabolomics, glycomics and lipodomics that provide increased sensitivity, specificity, and robustness over traditional methods.

<sup>5</sup> See “Microbial Forensics Implementation Plan elements 2.1-2.9.

5. Develop a strategic roadmap for the integration of microbial forensics metadata into a unified, web based, relational information system that provides both researchers and operations personnel rapid access to robust microorganism details for forensics interpretation. Roadmap should focus on current systems candidates, integration specifications, requirements data, life cycle hosting IT support, and management concept.
6. Develop statistical approaches and bioinformatics models that are capable of incorporating diverse analytic results (genetic, phenotypic and physical/chemical) into forensic comparisons and building networks and models to help investigators draw inferences regarding sample relatedness with described confidence intervals.

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## **Goal II. Promote Interagency Communication, Coordination and Information Sharing on Microbial Forensics Research and Development.**

In addition to a strong research agenda to develop microbial forensic methods, the second goal of this strategy is to facilitate interagency cooperation to spur the development of the field as a whole. Interagency communication, coordination, and collaboration will be the key to developing an enduring national capability in microbial forensics to support attribution investigations. A diverse group of federal stakeholders have a shared interest in the development of microbial forensics to support a range of attribution investigations and these shared interests and activities must translate to concerted action and cooperation. The developing field of microbial forensics faces broad scientific challenges that require sustained research and resource commitment. Only strong interagency partnerships will ensure the development of a powerful microbial forensic investigative capability to support attribution investigations. Microbial forensics stands to benefit by leveraging existing government biodefense programs wherever relevant. Assessing the areas of opportunity that could be leveraged for microbial forensic development is an important part of the interagency collaboration that is needed.

**Objectives:** Improve interagency communication, coordination, and information sharing. A.) establish and draft terms of reference and a charter for a formal Interagency Microbial Forensics Advisory Board (IMFAB) and B.) develop a National Archive and Resource for Forensically-Important Pathogen Strain Collections to serve microbial forensics.

### **A. Establish and draft terms of reference and a charter for a formal Interagency Microbial Forensics Advisory Board (IMFAB). The IMFAB shall:**

1. Identify the microbial forensic analytical requirements from relevant government stakeholders to support attribution investigations.

2. Review and update the National Strategy for Microbial Forensics Research and Development annually and align this strategy with microbial forensic analytical requirements from government stakeholders.
3. Develop an agreed upon interagency lexicon of microbial forensics terms based upon current understanding and state of the art in the field that is to be published as a position paper with the lexicon.
4. Support coordination and collaboration on microbial forensics research efforts.
5. Guarantee high quality research and development
6. Establish and sponsor an in depth periodic interagency peer review mechanism to complement the research agenda and inform resource allocations relevant to the strategy. The IMFAB shall select the members of the review panel to include government scientists and non-government scientists with the appropriate clearance levels to participate in these reviews.
7. Design and sponsor periodic objective capabilities assessments based on interagency exercises that incorporate realistic scenarios and sample sets to generate forensic data from which inferences can be drawn and be graded against the scenario.
8. Provide recommendations and expert advice to the National Archive and Resource for Forensically Important Pathogen Strain Collections.
9. Conduct professional outreach and engage existing interagency and outside scientific working groups and professional societies, as needed, to further the development of microbial forensics capabilities.

**B. Develop a National Archive and Resource for Forensically-Important Pathogen Strain Collections to serve microbial forensics.**

1. Convene interagency stakeholders to identify their requirements for a National Archive and Resource for Forensically – Important Pathogen Strain Collections and coordination with existing collections of microorganisms.

2. Develop a national pathogen strain management system that uses existing resources and paradigms, in as much as possible, to best serve the interests of stakeholder agencies and ensure the fullest participation of the biodefense, research, and microbial forensics communities.
3. Establish a Microbial Forensic Resource Center (MFRC) that can support the requirements of assay development and validation by providing reference standards as per Goal I, Objective B.6 of the Research and Development Strategy.

### **Goal III. Develop Effective Interagency Education and Training on Microbial Forensics Designed to Inform Policymakers and Scientific and Technical Personnel**

In order to better inform national security professionals, policymakers, the analytic and interested scientific communities to the complexities of microbial forensic analysis, a system of education and training is required. The educational focus will occur at two levels. The first level of education would consist of a broad overview for those individuals for whom an awareness of important forensic issues and their implications is all that is required. The next level of training shall focus, in depth, on the scientific challenges and complexities inherent to forensic analysis of microorganisms. Together both levels of education will form a core curriculum that will inform consumers and serve to produce a more informed core of analytic professionals.

**Objectives:** Establish and develop a core curriculum of training courses that will provide: A.) high level overview of the microbial forensics discipline and the challenges of forensic analysis and B.) “in depth” technical courses on the types of analyses used and the limitations and challenges inherent to them.

**Actions:**

1. Develop a core course which introduces the field of microbial forensics at a high level, appropriate for non-technical professionals.
2. Develop a core training program on specific technical topics relevant to microbial forensics.

3. Develop and deliver courses in microbial forensics relevant topics, such as, PCR methods, next generation sequencing technology, comparative genomics, bioinformatics and microbial ecology.