Chemical and Physical Signatures for Microbial Forensics
State of Microbial Forensics and Future Directions

Microbial forensics is a burgeoning field which unites biological, physical, and chemical disciplines for the forensic characterization of evidence with the goal of biocrime and bioterrorism attribution. One of the ongoing challenges facing this field is the vast diversity of possible biothreat agents. Over 1,000 naturally occurring disease agents are known to infect humans, including 217 types of viruses, 538 bacterial species, 307 fungal species, and 66 species of parasitic protozoa. Within each of these biological groups, numerous variants also exist. Another grand challenge is determining the limits of what science and technology can contribute to precise understanding of the provenance of specimens with this diverse array of possible threat agents that could be used as weapons.

To study the multitude of biothreats, microbial forensics has been particularly reliant on phylogenetic tools to examine the natural diversity of pathogenic microbes and their near neighbors. Despite these efforts, it is unlikely that the level of discrimination and precision with microbial genetics will achieve the standards set by human DNA forensics. In the best case, genetic analysis can exclude sources, narrow the population of possible sources, and support associations with potential sources. To complement these genetic techniques, chemical and physical methods have been and are being developed to compare relevant signatures imparted to microbial samples through their growth and post-growth environments and processing. Deliberate production of a microbial agent could involve several steps, each of which could vary in its nature. The variability inherent in production methods could clearly impart a wide range of phenotypic and processing signatures to the final product. Physical and chemical methods could thus provide significant comparative characterization power and augment phylogenetic analyses of biological agents for more exploitative and informative results and conclusions.

Developing forensic capabilities that incorporate the full suite of genetic, physical, and chemical methods currently available will be a complex and challenging task requiring sustained cooperation between scientists in many disciplines and those who use forensic information. In the last several years,
scientists, program managers, and consumers have come together in scientific working groups to generate forensic guidelines and evaluate promising methods. However, no integrative strategy for the analysis of biothreat agents currently exists. To bring microbial forensics to the desired level of maturity and defensibility, we must base newly developed technique on both sound scientific principles and a deep understanding of investigative legal, intelligence, and policy requirements.

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We live in a scientific age with unprecedented access to the microbial cell. Biotechnological advances have allowed us to transform entire genomes, alter intracellular chemistries, and express exotic compounds, all with relative ease. Yet, as the facility with which we can grow and manipulate microorganisms in the laboratory increases, so too does the possibility of harnessing microbes for illicit activities. Within the United States, incidents like the intentional *Salmonella* poisoning in The Dalles, Oregon, in 1984 and the *Bacillus anthracis* mailings in September–October 2001 underscore the ongoing threat of microbially mediated bioterrorism and its potentially devastating consequences.

In response to this threat, the field of microbial forensics has emerged to develop analytical techniques for processing microbiological evidence and extracting signatures that can help in the attribution of a biocrime. Up to now, much of this signature research has focused exclusively on the genotypic variation of biothreat agents. While genetic characterization can be a powerful investigative tool, interpretation of genetic markers is challenged by the complicated phylogenies of certain pathogens and the possibility of genetically identical strains existing in multiple laboratories. Microbial phenotypes are intrinsically dynamic systems, with properties that change in response to external stimuli as the microorganism grows and adapts to its culturing conditions. In this way, microbes can act like data loggers – recording physical and chemical signatures within the cell that reflect the methods that were used to prepare the organism and potentially capturing differences in the culturing procedures that may exist between laboratories or even individual scientists.

It is this latter area of microbiological signatures in which this book is framed. With it we hope to acquaint the reader with the existing state of research for a variety of different phenotypic and trace signature systems associated with cultured microorganisms. The topical breadth of chapters reflects the truly interdisciplinary nature of this field.

As with most nascent fields, phenotypic signature research offers many unique challenges, the most significant of which is transitioning from the auspices of academic inquiry into the courtroom where it may withstand the scrutiny of evidentiary standards. A recent report issued by the National Academy of Sciences emphatically highlighted the weaknesses of many
current forensic techniques and the need for new methods to have strong statistical foundations that are relevant to the courtroom.

*With the exception of nuclear DNA analysis, however, no forensic method has been rigorously shown to have the capacity to consistently, and with a high degree of certainty, demonstrate a connection between evidence and a specific individual or source.*

...The simple reality is that the interpretation of forensic evidence is not always based on scientific studies to determine its validity. This is a serious problem. Although research has been done in some disciplines, there is a notable dearth of peer-reviewed, published studies establishing the scientific bases and validity of many forensic methods. (*Strengthening Forensic Science in the United States*, S-5, S-6; 2008)

It is for this reason that we begin our book with a chapter on the statistical framework for microbial forensics. Even though many of the signature systems presented here are only in development or the initial stages of forensic application, it is imperative that experimental design and the statistical implications be considered during the development of any forensic method. In the same vein, we end the volume with a chapter on procedures for assuring the quality of operations and analyses in a forensic laboratory. This is a description of the rigorous scrutiny needed to ensure that forensic testing results are reproducible and defensible. It serves as a clear reminder of the context in which true forensic assays are ultimately used.

We hope that *Chemical and Physical Signatures for Microbial Forensics* serves as an accessible resource to educate readers about the forensic potential of various chemical and physical signatures that microbial cells and cultures incorporate during growth and post-growth processing, the power of integrating those signatures, and also to highlight the requirements of any assay if it is ultimately to be used in a forensic setting.

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