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Education and training in microbial forensics

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Microbial forensic research has led to the development, expansion, and evolution of new technologies, tools, and analytical capabilities bolstering our collective preparedness and response. A parallel commitment to education and training in this field is needed to prepare the next generation of scientists. The scientific bases, advances, applications, interpretations, and lessons learned by those who have been intimately involved in the early years of microbial forensics need to be documented and transferred to the next generation of scientists and decision makers so that society can be better protected from potential harm resulting from acts of bioterrorism and biocrime. An understanding of the microbial forensic field is essential to determining what evidence is collected, what proper and safe methods of collection and preservation to

employ, how the evidence is analyzed, what the significance of a result is, and what is supportive in identifying a perpetrator for prosecution. Thus, the burgeoning field of microbial forensics should be accompanied by a concomitant development of educational infrastructure and resources targeted at the next generation of practitioners, as well as diverse elements for the policy, research, and law enforcement communities.

A microbial forensic education program can take many forms depending on its purpose and target audience. It can be broad, providing information encompassing all aspects of the field from science to policy, or more focused. On one end of the target audience spectrum is the student at an academic institution who desires to enter into the discipline of microbial forensics and would like to be well prepared for a career choice. Students

following a broad academic education and training pathway have many options. They may, for example, (1) become forensic scientists analyzing crime scene evidence for law enforcement or intelligence agencies; (2) work as a research scientist in a government laboratory such as the CDC, FBI, USDA, or EPA; (3) work as a scientist in an industrial or commercial setting; or (4) enter graduate school toward a career in academia. Alternatively, the student may become an investigator who employs traditional law enforcement approaches with the most effective evidentiary documentation, collection, and preservation techniques merged with those of epidemiology for attribution purposes or crime investigation (Budowle et al., 2007). An individual may become a law enforcement official whose responsibility is to understand the scope of an investigation and what tools are available to generate investigative leads. Policy makers must have a general understanding of microbial forensic results, keep abreast of technological and analytical advances, and develop better appreciation of their implications to effect sound and defensible policy decisions. Finally, an important group that informs the public and government is the news media. They are frequently the primary interface between the scientist and the public, publishing and reporting their observations, insights, or inaccuracies of great importance and impact. Social media avenues have dramatically increased the speed with which news stories, factual or erroneous, are promulgated to the public. Educational efforts will better prepare such individuals to be informed and responsible and must be varied in depth and scope to match the target audience of various entities involved in microbial forensics.

There can be many formats and venues for microbial forensic education. Full academic-style programs should be developed at universities to comprehensively educate individuals in this applied science. Microbial forensics will necessarily cover a broad range of topics (microbiology, epidemiology, evolution, forensic science, genetics, statistics, infectious diseases, etc.), and no one can be

an expert in all aspects. However, all interested parties need to have some requisite, core knowledge in the various aspects of the discipline.

There are currently no full academic undergraduate (UG) or graduate degree programs in microbial forensics as of the writing of this chapter to the best of our knowledge. However, there are academic programs in microbiology, molecular biology, and forensic science, and related topics, with affiliated courses and training opportunities that provide avenues and strategies for the preparation of students. Additionally, a full academic program likely is not practical for working professionals; more abbreviated educational/training activities, similar to that of a continuing education course format, could be very effective at integrating professionals into the microbial forensic discipline. Microbiologists, epidemiologists, public health, and law enforcement officials are highly trained in relevant aspects of microbial forensics but may need additional training to integrate effectively their expertise with the demands of this new discipline. Shorter courses or symposia will be useful for expanding the knowledge base of trained professionals. To broadly educate and to specifically educate those involved in the widely varied aspects of microbial forensics represents an educational challenge that must be met to develop the experts and expertise that we desperately need to combat bioterrorism and biocrime. Microbiology, epidemiology, and forensic science education are all at the heart of this field. Program directors, educators, and trainers may review the recommendations and resources from academic, government, and professional organizations in these and related areas. For example, the American Society of Microbiology (ASM) Education Board on microbiology education ([asm](#)) has developed and published many educational resources that may be useful for developing training strategies. The ASM proposed a document on Vision and Change in microbiology education ([vision and change](#)) with five recommended overarching microbiology curriculum concepts: (1) Evolution;

(2) Cell Structure and Function; (3) Metabolic Pathways; (4) Information Flow and Genetics; (5) Microbial systems; and a sixth applied concept, the Impact of Microbes. The new curriculum guidelines were derived to be in line with recommendations from the American Association for the Advancement of Science Vision and Change education publication ([American Association for the Advancement of Science \(AAAS\), 2011](#))

Resources from the American Academy of Forensic Sciences ([aafs](#)) for forensic science education tools and opportunities are available. These include the Forensic Education Program Accreditation Commission recommendations for forensic science coursework, electives and laboratory courses fit to specific subdiscipline areas ([fepac](#)), a Young Scientists Forensic Forum providing a forum for student-led seminars, research, and networking ([yfsf](#)), an International Education Outreach program ([international](#)), and an annual Forensic Science Educators Conference targeted at high school teachers ([forensic](#)). Microbial forensic educators and trainers may find these resources useful. These ASM and AAFS resources are dedicated to educating future scientists and contain recommendations for core, elective, and required laboratory courses, preparation on legal issues, seminars, public speaking, and court testimony. Other useful groups include the Council of Forensic Science Educators ([cofse](#)), the National Forensic Science Technology Center ([nfstc](#)), the National Clearinghouse for Science, Technology and the Law ([ncstl](#)), and the Organization of Scientific Area Committees for Forensic Science Interdisciplinary Virtual Subcommittee on Training ([nist](#)) that all provide educational tools and resources in forensic science. Additional resources may be found in reports and conference proceedings on biocrimes, defense, and biothreat agents ([disarmament](#)) that may be available from DHS, CDC, and DoD, for example ([frontiersin](#)).

A list of topics in [Table 32.1](#) covers the spectrum of educational opportunities in microbial forensics and could form the template for a

comprehensive education and training program. Clearly some areas are more relevant to scientists, others to crime scene investigators, and others to decision makers. Many of the subjects naturally overlap. Some general areas are identified and discussed briefly as potential parts of the core curriculum for scientists. Most of these topics are addressed in greater detail in other chapters of this book.

Microbial forensic curricula and training

Microbial forensics is defined as a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime, or inadvertent release of a microorganism/toxin for attribution purposes ([Budowle et al., 2003](#)). It is the same as other forensic disciplines except for its focus on a particular type of crime ([Budowle et al., 2003](#); [Carter et al., 2017](#)). Based on past history and with current technology capabilities, the potential use of biological weapons is greater than at any other time in history. Only a few semiexpert individuals are needed with access to dual-use equipment (e.g., equipment used in the pharmaceutical or food industries) to produce bioweapons inexpensively. These bioweapons will contain signatures that might be exploited to help identify the perpetrators. One may consider attribution solely to be the “DNA fingerprinting” of a pathogenic agent, but unique genomic identification of a microorganism may not always be possible because of the clonal nature of many microorganisms and, on a case-by-case basis, lack of population and phylogenetic data.

Microbial forensics employs the same general practices as other forensic disciplines. Recognizing a crime scene, preserving a crime scene, chain of custody practices, evidence collection and handling, evidence shipping, analysis of evidence, and interpretation of results are carried out in the same general manner as other forensic evidence. A common exception is that evidence will be handled as a biohazard (even

TABLE 32.1 Overview and origins of microbial forensics.

Basic epidemiology
Molecular epidemiology
Microbial forensic curricula and training
Basic and advanced
Microbes and their products
a. Viruses
b. Bacteria
c. Fungi
d. Eukaryotic parasites
e. Toxins
The host target—how does a person or animal become ill?
Immunology
The plant as a target—how does a plant or crop get damaged?
The host response as a forensic indicator
a. Immune system
b. Pharmacokinetics
c. Antibiotics
Processes and technology
a. Sample collection
b. Forensic handling
c. Preservation
d. Extraction
e. Advanced microscopy
f. Proteomics
g. Genomics
h. Bioinformatics
i. Statistical analysis and confidence estimations
j. Indicators of engineering
k. Synthetic biology
l. Population genetics
m. High-throughput sequencing
n. Nonbiologic tools
o. Sensitive signature detection and characterization
p. Evolving, nascent technology
Quality assurance and quality control
Investigative genetics (i.e., forensic genetics)
a. Interpretation
b. Forensic science in general
Crime scene investigation
a. Identify crime scene
b. Evidence collection
c. Sampling strategies
d. Sample storage and transportation
e. Trial preparation including moot court
Case histories
a. Civilian
i. Food safety and public health
1. Foodborne— <i>Shigella</i> , <i>Salmonella</i> (spinach)
2. Anthrax
3. Ricin

TABLE 32.1 Overview and origins of microbial forensics.—cont'd

<ul style="list-style-type: none"> ii. Agriculture <ul style="list-style-type: none"> 1. Foot-and-mouth UK 2007 2. Mad cow disease US 2003 (attribution by host genetics) iii. Environmental science <ul style="list-style-type: none"> Poultry industry water contamination Arkansas iv. Emerging infections <ul style="list-style-type: none"> 1. H1N1 2. Severe acute respiratory syndrome 3. Monkeypox 4. HIV b. Criminal <ul style="list-style-type: none"> i. US anthrax 2001 with focus on technology and investigation HIV ii. Ricin c. Biodefense <ul style="list-style-type: none"> Terrorism and biocrimes
Legal issues
<ul style="list-style-type: none"> a. United States b. International
Select Agent rules
Operational and intelligence issues
National-level capabilities and resources
a. Country capabilities <ul style="list-style-type: none"> i. What and how should any country be prepared? ii. What strategies make sense? iii. Planning, implementing, and measuring effectiveness iv. Exercises v. Where can additional support be sought? vi. Epidemiologic investigation as a basic country skill
Public information (media and public) <ul style="list-style-type: none"> Dissemination of accurate information in timely manner
Entertainment industry <ul style="list-style-type: none"> Depiction of accurate information

more so than, for example, HIV-infected blood). It is anticipated that the majority of microbial forensic evidence will fall into a category with shared characteristics, with some data being very informative and some being less informative. An understanding of the field is essential to determining what type of evidence is collected, how it is analyzed, what the significance of a result is, and what is supportive in identifying a perpetrator and for prosecution.

To support a career in microbial forensics, a university microbial forensic curriculum will necessarily cover a broad range of disciplines, which

may include microbiology, chemistry, statistics, epidemiology of infectious diseases, evolution, genetics, genomics, and forensics. These courses could be taught individually or merged into a few dedicated microbial forensic courses. From a practical standpoint, many microbial forensic training programs will be based in other majors or minors in epidemiology, genetics, molecular biology, or microbiology as there are numerous graduate programs already in place for epidemiology and these other fields ([publichealthonline](#)).

A major in epidemiology or microbiology could easily become a training platform for microbial

forensics with the addition of select courses that include fundamentals in forensics. Alternatively, a forensic science program with additional training in basic sciences such as microbiology and epidemiology could serve to educate microbial forensic scientists. It will be important to emphasize integration of the material toward a specific microbial forensic profession. Concurrent enrollment in microbial forensic seminars, capstone courses, and internships will be needed to provide students the contextual importance of the basic material toward their chosen discipline that will often be taught more generically or under an unrelated discipline.

Research internships and forensic seminars provide important opportunities to conduct hands-on experimentation, analyses, and data interpretation and exposure to recent advances in the field. Seminars also provide a forum for student research presentations and public speaking. External workshops, conferences, and meetings ([index](#); [scientific](#); [meetings](#)) augment opportunities to present research, gain further insights, network, and to become aware of emerging advances in the field from microbial forensic experts. Students that present and publish their results contribute to moving the field forward and advancing their careers. Training in the communication of science is a pivotal aspect of preparing our future science leaders and should be an integral part of any microbial forensic education program.

Curricular guidelines from the American Society of Microbiology and American Academy of Forensic Sciences

Recent national reports have addressed the need for changing how science courses in higher education are taught, so that students develop a deeper understanding of critical concepts and the analytical and cognitive skills needed to address future challenges. Our competitiveness and national success depend on our students'

science aptitude ([edsource](#)). National Science Teachers Association teachers point to lack of student motivation as a major concern in US science education ([news](#)). The need for a new approach to improve science and math education is compelling and well supported. In the American Academy for the Advancement of Science Project 2061, "just doing more science was not the answer" ([project 2061](#)). Current methods put more value on learning correct answers versus exploration, collaboration, and inquiry. They recommend "practices where the learning of science is as much about the process as the result or outcome, and where students can ask questions and are actively engaged in the learning process, refocusing student learning from knowledge and comprehension to application and analysis." ([project 2061](#)). Inquiry is "central to science learning" as students develop their understanding of science concepts combining knowledge, reasoning, and thinking while enhancing comprehension through hands-on learning. The National Research Council agrees. They state "effective instruction capitalizes on students' early interest and experiences, identifies and builds on what they know, and provides them with experiences that serve to engage them in the practices of science and sustain their interest" ([NRC, 2011](#)).

Because discipline-specific professional societies have national stature and are often the organizations that set guidelines or standards within a discipline, they are well-suited to play a role in promoting systemic change. In 2010, the AAAS and the NSF released the report entitled "Vision and Change in Undergraduate Biology Education: A Call to Action" ([American Association for the Advancement of Science \(AAAS\), 2011](#)). In response to these recommendations, the ASM revised its curriculum guidelines for introductory microbiology courses to emphasize a deeper "understanding of core concepts, critical thinking, and essential laboratory skills." ([American Association for the Advancement of Science \(AAAS\), 2011](#)).

In 2012, the ASM Education Board published curriculum guidelines and recommendations for education in microbiology (Merkel & the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology, 2012).

“These Guidelines incorporate many of the recommendations made in Vision and Change. They embrace the scientific process and thinking skills put forth in Vision and Change, adding microbiology-based laboratory skills. Further to adopting the five core concepts of Vision and Change, the Guidelines added a sixth core concept, Impact of Microorganisms, specific to this field. Each of the six core concepts is exemplified by four or five microbiology-specific fundamental statements, which reflect basic concepts that are important for all microbiology students to understand in depth. These 27 fundamental statements, together with the four scientific thinking skills and seven laboratory skills, form a comprehensive framework for an undergraduate microbiology course. (Merkel and the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology, 2012). In addition, the ASM Curriculum Guidelines were designed to focus microbiology teaching on student-centered goals and priorities and to enable educators to adopt the discipline-based approach to course design for microbiology courses.”(Horak et al., 2015).

The American Society for Microbiology has developed a number of educational programs and resources with student-centered learning modules that may be useful in developing forensic microbiology education programs (Merkel, 2016).

The resources cited above are codified in Table 32.2. (Merkel & the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology, 2012), 32.3 (Merkel, 2016), and 32.4. (Merkel, 2016) (Tables 32.3 and 32.4).

Additional guidelines have also been published for Nursing and Allied Health (McKay and ASM MINAH Undergraduate Curriculum Guidelines Committee, 2018).

The Undergraduate Education Committee has developed a set of curriculum guidelines for microbiology majors in “ASM’s curriculum recommendations: Microbiology Majors Program”(Emmert and the ASM Task Committee on

Laboratory Biosafety, 2013). In this document, ASM provides “recommendations for conceptual knowledge, recommended core and elective courses, and laboratory skills and safety as well as issues for further action and discussion ... to be used by programs in their own assessment, maintenance, and formation of strong programs in microbiology.” Table 32.5 contains the recommendations for UG microbiology education and core and elective courses, and Table 32.6 lists recommendations for laboratory skills, laboratory safety guidelines from ASM have also been published and are listed for BSL1 and BSL2 levels (Emmert and the ASM Task Committee on Laboratory Biosafety, 2013).

These recommendations provide a starting framework for developing curriculum requirements fit to the goals of programs and the target students they serve.

ASM prompted further discussion in its curricular recommendations stating “A major problem noted was time and resources. The list is long and it is not possible to fit all of these courses into a 4-year program nor are all courses appropriate for all students. A critical component to this issue may be in getting the students into the major early. The Introduction to Microbiology course should be designed so that it can be taken no later than the fourth semester (end of second year) of study. This will allow for two years of advanced study of microbiology” (Emmert and the ASM Task Committee on Laboratory Biosafety, 2013).

Another potential solution would be to require students to double major. In this dual BS model, students would be required to complete two BS degrees. Core science and laboratory courses would be covered in the first BS science degree (to fit the target career trajectory). The BS in Microbial Forensics specialty area would therefore have additional coursework space for upper division science and elective laboratory courses. There are a few forensic science programs that use this model such as the University of Central Oklahoma.

TABLE 32.2 ASM Curriculum Guidelines: list of recommended skills, core concepts, and fundamental statements.

Evolution

1. Cells, organelles (e.g., mitochondria and chloroplasts), and all major metabolic pathways evolved from early prokaryotic cells.
 2. Mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms.
 3. Human impact on the environment influences the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).
 4. The traditional concept of species is not readily applicable to microbes due to asexual reproduction and the frequent occurrence of horizontal gene transfer.
 5. The evolutionary relatedness of organisms is best reflected in phylogenetic trees.
-

Cell structure and function

6. The structure and function of microorganisms have been revealed by the use of microscopy (including bright field, phase contrast, fluorescent, and electron).
 7. Bacteria have unique cell structures that can be targets for antibiotics, immunity, and phage infection.
 8. Bacteria and archaea have specialized structures (e.g., flagella, endospores, and pili) that often confer critical capabilities.
 9. While microscopic eukaryotes (for example, fungi, protozoa, and algae) carry out some of the same processes as bacteria, many of the cellular properties are fundamentally different.
 10. The replication cycles of viruses (lytic and lysogenic) differ among viruses and are determined by their unique structures and genomes.
-

Metabolic pathways

11. Bacteria and archaea exhibit extensive, and often unique, metabolic diversity (e.g., nitrogen fixation, methane production, anoxygenic photosynthesis).
 12. The interactions of microorganisms among themselves and with their environment are determined by their metabolic abilities (e.g., quorum sensing, oxygen consumption, nitrogen transformations).
 13. The survival and growth of any microorganism in a given environment depends on its metabolic characteristics.
 14. The growth of microorganisms can be controlled by physical, chemical, mechanical, or biological means.
-

Information flow and genetics

15. Genetic variations can impact microbial functions (e.g., in biofilm formation, pathogenicity, and drug resistance).
 16. Although the central dogma is universal in all cells, the processes of replication, transcription, and translation differ in bacteria, archaea, and eukaryotes.
 17. The regulation of gene expression is influenced by external and internal molecular cues and/or signals.
 18. The synthesis of viral genetic material and proteins is dependent on host cells.
 19. Cell genomes can be manipulated to alter cell function.
-

Microbial systems

20. Microorganisms are ubiquitous and live in diverse and dynamic ecosystems.
 21. Most bacteria in nature live in biofilm communities.
-

TABLE 32.2 ASM Curriculum Guidelines: list of recommended skills, core concepts, and fundamental statements.—cont'd

-
22. Microorganisms and their environment interact with and modify each other.
23. Microorganisms, cellular and viral, can interact with both human and nonhuman hosts in beneficial, neutral, or detrimental ways.
-

Impact of microorganisms

-
24. Microbes are essential for life as we know it and the processes that support life (e.g., in biogeochemical cycles and plant and/or animal microbiota).
25. Microorganisms provide essential models that give us fundamental knowledge about life processes.
26. Humans utilize and harness microorganisms and their products.
27. Because the true diversity of microbial life is largely unknown, its effects and potential benefits have not been fully explored.
-

Downloaded from Susan Merkel* and the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology (2012). *The Development of Curricular Guidelines for Introductory Microbiology that Focus on Understanding*. *J Microbiol Biol Educ.* 2012; 13(1): 32–38. Published online 2012 May 3
 Downloaded from https://www.asm.org/getattachment/1b074b9e-8522-4d9d-bbc3-c0ca9b9abf1a/FINAL_Curriculum_Guidelines_w_title_page.pdf.

TABLE 32.3 Examples of lower-order and higher-order learning outcomes and assessments from some ASM fundamental statements.

Example core concept and fundamental statement	Example lower-order learning outcome: after this unit, students should be able to	Example higher-order learning outcome: after this unit, students should be able to
<i>Evolution</i> Mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms.	... describe three mechanisms of horizontal gene transfer in bacteria.	... interpret sequence data to determine if horizontal gene transfer has occurred.
<i>Cell structure and function</i> The structure and function of microorganisms have been revealed by the use of microscopy.	... explain how the cell structure of gram-negative and gram-positive cells leads to a given gram stain result.	... compare and contrast the effects of doing the gram stain incorrectly on gram-negative and gram-positive bacteria.
<i>Metabolic pathways</i> Bacteria and archaea exhibit extensive, and often unique, metabolic diversity.	... draw a diagram that shows the process of nitrogen fixation in cyanobacteria.	... design a mechanism that would allow a bacterium to protect its nitrogenase from oxygen.
<i>Information flow and genetics</i> Genetic variations can impact microbial functions.	... identify each of the following: point mutation, genetic insertion, genetic deletion and frameshift mutation.	... predict whether or not a given mutation (genotypic change) would result in a change of function (phenotypic change).
<i>Microbial systems</i> Most bacteria in nature live in biofilm communities.	... order the stages of biofilm formation and maturation.	... develop a drug that would prevent biofilm formation.
<i>Impact of microorganisms</i> Because the true diversity of microbial life is largely unknown, its effects and potential benefits have not been fully explored.	... measure cell density using viable cell counts and microscopy methods and explain the differences.	... propose an experiment that would allow you to prospect for antibiotics in a new environment.

From: Merkel, S., August 2016. *FEMS Microbiol Lett.* 363(16), pii: fnw172. <https://doi.org/10.1093/femsle/fnw172>. Epub July 12, 2016. American Society for Microbiology resources in support of an evidence-based approach to teaching microbiology: Downloaded from <https://academic.oup.com/femsle/article/363/16/fnw172/2197755> and content available at <https://www.asm.org/ASM/media/Education/FINAL-Learning-Outcomes-w-title-page.pdf>.

TABLE 32.4 List of ASM resources that support evidence-based teaching and learning.

Resource	Description	Website
ASM Curriculum Guidelines for an Undergraduate Microbiology Course	Concepts and competencies for an introductory undergraduate microbiology course	https://www.asm.org/index.php/guidelines/curriculum-guidelines
Learning outcomes for the ASM Curriculum Guidelines	Examples of lower-order and higher-order learning outcomes	https://www.asm.org/index.php/guidelines/curriculum-guidelines
ASM Sample Questions in Microbiology (release in 2016)	Collection of peer-reviewed multiple-choice and true/false questions	http://www.asmscience.org
Microbiology Concept inventory and Microbiology for Health Sciences Concept inventory (release in 2017)	Tested questions developed to assess how well students understand critical concepts	facultyprograms.org/index.php/resources/concept-inventories
Journal of Microbiology and Biology Education	Open access, peer-reviewed collection of research articles and activities	http://www.asmscience.org/content/journal/jmbe
MicrobeLibrary	Peer-reviewed visual resources and laboratory protocols	https://www.microbelibrary.org
ASM Faculty Programs	Portal to the ASM educational resources	http://www.facultyprograms.org
ASMCUE	Interactive 4-day conference for biology educators	http://www.asmcue.org
Biology scholars Program	Five-month hybrid courses offering a range of training in microbiology education	http://www.facultyprograms.org/index.php/biology-scholars-hybrid-courses
Science Teaching fellowship Program	Five-month online program to prepare doctoral-trained students for science teaching positions	http://facultyprograms.org/index.php/stf-program
ASM webinars	Online courses on teaching and research	http://www.facultyprograms.org/index.php/webinars
Guidelines for biosafety in Teaching laboratories	A comprehensive guidebook of best practices for safely handling BSL-1 and BSL-2 microbes in teaching labs.	https://www.asm.org/index.php/guidelines/safety-guidelines

Downloaded from Merkel S., August 2016. *FEMS Microbiol Lett.* 363(16), pii: fnw172. <https://doi.org/10.1093/femsle/fnw172>. Epub July 12, 2016. American Society for Microbiology resources in support of an evidence-based approach to teaching microbiology. <https://academic.oup.com/femsle/article/363/16/fnw172/2197755>.

Another issue that the ASM membership identified is the recommendation that the core courses all have labs. Laboratory courses are by their nature expensive. ASM raised the following question: “How can departments with limited resources deal with this resource problem?” One suggestion is to offer a two-semester sequence of independent lab courses that teach the skills

needed to be a microbiologist rather than offer a lab with every course. This approach may reduce the current recommendation of five lab courses in the core curriculum to two semesters of independent lab courses that cover the basic skills and one advanced course with laboratory (Emmert and the ASM Task Committee on Laboratory Biosafety, 2013).

TABLE 32.5 Recommended core and elective courses from the ASM Curricular Guidelines.

Core courses for microbiology majors

- Introduction to microbiology (with lab)
 - Microbial physiology (with lab)
 - Microbial genetics (with lab)
 - Microbial diversity and ecology (with lab)
 - One advanced course that includes laboratory
 - Capstone course (e.g., senior seminar presentation, independent research project, internship)
-

Elective courses for microbiology majors

- Immunology
 - Pathogenic microbiology
 - Food and dairy microbiology
 - Environmental microbiology
 - Marine microbiology
 - Industrial and applied microbiology
 - Biotechnology
 - Bioinformatics
 - Virology and other acellular agents
 - Parasitology/protozoology
 - Mycology
 - Phycology
 - Epidemiology
 - Public health
 - Undergraduate research and internship
 - Careers in microbiology^a
 - Bioethics^a
-

Support courses for microbiology majors

- General biology with lab (1 year)
 - Cell and molecular biology
 - General chemistry with lab
 - Organic chemistry with lab
 - Biochemistry (1 semester)
-

Continued

TABLE 32.5 Recommended core and elective courses from the ASM Curricular Guidelines.—cont'd

-
- Math (through calculus)
 - Physics (1 year)
 - Statistics (1 semester)
 - Scientific writing and technical communication
-

^a Bioethics and careers in microbiology could be integrated into core course material to ensure exposure of all students to the topics. Downloaded from Merkel S., the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology, 2012. The development of curricular guidelines for introductory microbiology that focus on understanding. *J. Microbiol. Biol. Educ.* 13(1), 32–38. Published online May 3, 2012 Downloaded from https://www.asm.org/getattachment/1b074b9e-8522-4d9d-bbc3-c0ca9b9abf1a/FINAL_Curriculum_Guidelines_w_title_page.pdf. Also available from- <https://www.asm.org/Articles/Education/ASM-Recommended-Curriculum-for-Microbiology-Majors>.

TABLE 32.6 Recommended scientific thinking and laboratory skills from ASM curriculum recommendations.

Scientific thinking

1. Ability to apply the process of science
 - a. Demonstrate an ability to formulate hypotheses and design experiments based on the scientific method.
 - b. Analyze and interpret results from a variety of microbiological methods and apply these methods to analogous situations.
 2. Ability to use quantitative reasoning
 - a. Use mathematical reasoning and graphing skills to solve problems in microbiology.
 3. Ability to communicate and collaborate with other disciplines
 - a. Effectively communicate fundamental concepts of microbiology in written and oral format.
 - b. Identify credible scientific sources and interpret and evaluate the information therein.
 4. Ability to understand the relationship between science and society
 - a. Identify and discuss ethical issues in microbiology.
-

Microbiology laboratory skills

5. Properly prepare and view specimens for examination using microscopy (bright field and, if possible, phase contrast).
 6. Use pure culture and selective techniques to enrich for and isolate microorganisms.
 7. Use appropriate methods to identify microorganisms (media-based, molecular, and serological).
 8. Estimate the number of microorganisms in a sample (using, for example, direct count, viable plate count, and spectrophotometric methods).
 9. Use appropriate microbiological and molecular lab equipment and methods.
 10. Practice safe microbiology, using appropriate protective and emergency procedures.
 11. Document and report on experimental protocols, results, and conclusions.
-

Downloaded from <https://www.asm.org/ASM/media/Education/ASM-Curriculum-Guidelines.pdf>.

The depth of the curriculum will vary depending on the level and occupation of the student. High school students may have abbreviated versions that can pique their appetites to learn more. College students will need comprehensive training to prepare them for graduate school or for entering the workforce. Legal experts will require an overview to understand the limitations of the field and how to support or refute scientific findings.

Basic epidemiology

Epidemiology is a cornerstone of public health and is critical to microbial forensics. One goal of epidemiology is to recognize infectious disease outbreaks and to attribute the outbreak to a source to prevent additional cases (see Chapter 16 for further detailed information). In many aspects, microbial forensics employs the same

tools as those used in epidemiology. A training program in microbial forensics will parallel many parts of current programs in epidemiology. Models can be obtained from epidemiology curricula, and experience from natural outbreaks will help guide how microbial forensic scientists will perform investigations of biocrimes. Tracing the course of an outbreak will assist in identifying the index case, cause, and/or time of the outbreak. With many disease outbreaks, as well as cases of unusual infections (e.g., monkeypox), the recurring question will be: Is this a natural event or an intentional attack? Epidemiological factors will help distinguish between natural or intentional events and enable more effective responses in either event. A biocrime may be recognized through surveillance linking multiple unusual disease occurrences in contiguous or noncontiguous geographic areas. A microbial forensic investigation may be based on initial public health findings and then proceed further to address attribution to identify the perpetrator(s) of a biocrime or bioterrorist act.

Molecular epidemiology

Molecular epidemiology focuses on the contribution of potential genetic, identified at the molecular level, and environmental risk factors to the etiology, distribution, and prevention of disease within families and across populations (Riley, 2004; Eybpoosh et al., 2017; Jagielski et al., 2016) [(3) also see the Genomics section below]. The field provides a good example where application of newer technologies may help overcome many of the same problems encountered with traditional epidemiology with respect to study design and interpretation (4). Molecular tools can be employed to characterize and potentially individualize samples and isolates to address forensically relevant questions. This subdivision of epidemiology has special importance in microbial forensics because it is desirable to

determine the source of a particular microbe used in a crime. Highly discriminating assays can precisely identify strains and isolates, resulting in a more focused and effective investigation. These types of data could associate a sample with a single geographic area, even possibly a particular laboratory or flask, or with the specific conditions and nutrients used to culture the microorganism. Some of these aspects are discussed in the chapters on anthrax.

Microbes and their products as biological weapons

Agents that can be used in biocrimes span the microbial world of viruses, bacteria, fungi, eukaryotic parasites, and toxins. It is important to have a basic understanding of each type of microorganism to appreciate the factors that make a particular microbe a serious threat as a weapon. These factors include accessibility, stability, transmissibility, associated history with weapons programs, and the capacity to produce disease with transient or sustained consequences, including death. Different technologies are needed to culture bacteria and viruses, as they differ greatly in growth requirements. Indeed, some microbes are difficult or impossible to culture. Such information may help an investigator understand what microbes should be considered as a high threat and how they might have been used in a particular circumstance. A basic understanding of different microbial classes and their products would include human, animal, and plant pathogens (Schutzer et al., 2005; Schmedes et al., 2016; Teshome, 2016).

Host factors including immune responses

It is important to understand how the host responds to microbes and the unique signatures that can be found, including those in response

to exposure to a particular microbe or for timing the exposure to a pathogen [(Tomkovich and Jobin, 2016) and see Chapter 20]. For forensic purposes, an immediate goal is to distinguish a potential victim from a perpetrator and to distinguish between a natural or intentional event. A basic understanding of the immune system, how antibodies are generated, when different classes of antibodies appear, and what cell types and their signatures are generated may assist in criminal investigations.

Processes and technology

Sophisticated equipment (technology) that resides in the laboratory is only part of the process for obtaining reliable and meaningful information. The process begins with sample acquisition and proceeds with packaging, storage, and analysis and ultimately ends up with interpreting the results. All aspects are important and must be integrated effectively to have high confidence in results.

Crime scenes and chain of custody

After recognizing that a bioterrorist act or biocrime has occurred, defining the crime scene is the first important part of an investigation. Depending on the nature of the crime, there may be multiple crime scenes requiring different sets of skills, knowledge, equipment, and abilities (see Chapters 21 and 22 for more details). Once the crime scene(s) has (have) been identified and delimited, a plan is needed to properly collect and maintain integrity of the evidence that may be subsequently analyzed. Practices are needed to maintain the integrity and that minimize contamination of the evidence. The handling and storage of evidence is integral to minimizing degradation of the target analytes. Microbial contamination may be somewhat different from other types of contamination because the contaminating organisms can replicate thus confounding results. The nuances of a microbial forensic

investigation add a layer on top of traditional crime scene investigations, particularly because of the hazardous nature of the evidence. The need for proper documentation may seem obvious but it is a very important part of maintaining the integrity of the evidence. Crime scenes are chaotic and missteps can occur. To minimize missteps in handling documentation procedures should be established so the crime scene can be reconstructed at a later date for investigators or in a court of law. It is likely that biocrimes and acts of bioterrorism will add another dimension of complexity because (i) there is less experience in crime scene collection due to (fortunately) fewer cases, (ii) addressing the safety of victims will not be trivial, (iii) investigators will be wearing cumbersome but absolutely necessary personal protective equipment (PPE), (iv) the best approaches for collection and preservation of evidence may have to be determined at the scene given the limited extant information available, and (v) the response to biocrime events by using forensic science in an attribution involves a complex interplay among science, policy, law, law enforcement, public health, medical, and media communities (**bioattribution**) and may require several different areas of expertise and authority. This aggregation of diverse disciplines and professions, “brought together to develop an understanding and action plan in response to a suspicious event” is challenging in a domestic context, and even more formidable if an event has global implications (Bidwell et al., 2016). The historical knowledge of experts intimately involved in past events and their understanding of the dynamics of the interplay of science–law–bureaucracy–media are pivotal in developing effective and efficient responses for future attribution determinations as well as in the training of our students. Thus, crime scene investigation, safety, PPE, collection, chain of custody, and sharing important past casework experiences and examples of how to manage the interplay of the different disciplines and professionals should be included as essential parts of any curriculum.

The first responder community needs to be aware of the safety issues and the methods of collection because they may become involved in performing evidence collection. Laboratorians must understand these processes because better decisions can be made as to what evidence is pertinent for analysis. Lawyers and judges will want to understand the basics of chain of custody to be assured that acceptable handling methods have been exercised to maintain the integrity of the evidence. Those who will have contact with the crime scene, as well as those in the laboratory who require downstream interoperability of collected evidence, will have to learn basic do's and don'ts of crime scene investigation (United States Federal Bureau of Investigation Laboratory FBI, 1999) to effect a better systems-based process. Education about crime scene investigation will help ensure use of validated microbial identification practices that will collect the most pertinent evidence and will best preserve the integrity of the evidence for analysis in a forensic laboratory.

Training resources, tools, and opportunities that may be useful to educators are available from other dedicated academic, government, and private industry groups in many of the sub-topics listed in Table 32.1. For example, there are research papers, newsletters, webinars, seminars, and guidelines at CDC, the Biological Security Countering Weapons of Mass Destruction (CWMD) US Homeland Security Office and disaster, mortuary operations response team units (DMORT) (emergency; topic; Nolte, 2003; Preparedness), which may be useful to educators, trainers, and students depending on the goals of the training and target audience.

Sample collection and preservation of forensic evidence

One must understand the tools available to collect the sample as well as the limitations posed with a collection process or tool (see

Chapters 21 and 22 (*new Budowle et al., 2005, 2006)). While most approaches focus on collection tools, it is very important to consider sampling strategies to obtain the most relevant data, which involves strategic planning, logistics, and statistics. Conditions that are proper for collection and/or preservation of one microbe may be deleterious for another and, for that matter, to traditional forensic materials such as human DNA, fingerprints, and trace materials. For example, foodborne pathogens are particularly vexing; conditions that are intended to preserve the material may promote growth of natural bacteria in a food product, and this overgrowth may destroy or obscure the initial bio-weapon. Tools for collection need to be validated for efficient collection and for determining that they do not react with the target of interest. Tools developed for powder collection may be inefficient or ineffective for collecting plant material. Sample collection is not trivial and requires substantial consideration. Issues related to the practices of sample collection, handling, transportation, and storage of microbes in the investigation of biocrimes and guidelines for the collection of evidence by physicians and medical personnel from potential victims of bioterrorism have been published and are valuable for education and training programs (Schutzer et al., 2005).

The same issues about evidence collection will need to be applied to preservation processes. It is imperative to prevent further degradation of the evidentiary target once collected. Conditions for preservation apply for packaging and shipping, for maintaining of the evidence in the laboratory, and for postanalysis storage.

Extraction

Extraction efficiency, particularly of interest to the scientist, pertains to obtaining the highest quality and quantity yield as possible of the target of interest. Yield is related to the quantity

and purity of target and removal from the collection matrix. Targets can include cells, nucleic acids, proteins, nutrients, growth materials, and elements.

Advanced microscopy

Various forms of microscopy may be used to visualize the evidence. These tools may range from light microscopy to electron microscopy to atomic force microscopy and are available for characterization of a microbe. These approaches are rapid and can be used to identify candidate threats as well as to dismiss hoaxes.

Proteomics

Defining chemical and physical properties of a biological agent can provide information on how and when the agent was produced and can be used to determine if two microbial samples were produced by the same process. Proteomics is a comprehensive study of the protein composition of biological systems at a moment in time or at different stages of a microbe's growth. Many proteins are conserved and can be used for general identification, while other proteins may be expressed in response to environmental stimuli, growth state, or growth conditions. Protein profiling can provide information beyond genomic analysis about the conditions of the bioweapon before host exposure (Gil and Monteoliva, 2014).

Genomics

One of the fastest growing areas with implications for microbial forensics is genomics. More rapid and in-depth sequencing of microbes is possible today than it was a decade ago; sequencing and targeted methods such as those used in the investigation of the anthrax-letter attack seem almost antiquated today. Genomic analyses will continue to be essential in

identifying species, strains, isolates, and individual samples to assist in a microbial forensic investigation. (Schmedes et al., 2016; Budowle et al., 2017; Karlsson et al., 2013). The rapid expansion of sequencing capabilities, where sequencing some microbes within a day at very deep coverage, has raised the importance of genetic identification. It will likely be a mainstay of the microbial forensic investigation of any attack with any microbe. The cost of whole-genome sequencing has decreased at least 100-fold in just a few years. This technology has now matured into one of the methods of choice to examine the genetic structure of a particular pathogen and to identify signatures of forensic relevance. (Schmedes et al., 2016; Budowle et al., 2017; Karlsson et al., 2013) In addition, the legal profession should have a basic understanding of the capabilities and limitations of these technologies to be successful in the courtroom, just as has been necessary for human DNA forensics. Several chapters in this book expand on technology in detail.

Interpretation, statistical analysis, and confidence

Interpretation of results and the bases to support interpretation by scientists will be critical to the end-user stakeholders that rely on microbial forensic evidence for legal proceedings, for setting policy or responding to a threat or an attack. Interpretations could be as simple as positive or negative to very complex evaluations using limits of detection, complex algorithms for identifying. A host of answers and additional questions can arise from data interpretation.

Central to interpretation is, when possible, a statistical analysis of the findings which should be performed to provide significance of the result, uncertainty, or to convey the strength of the evidential results. A variety of statistical approaches exists, and it is imperative to understand which ones apply to particular analyses and interpretations.

One needs to consider that traditional statistical analyses may not apply to microbial evidence in some cases. With the advent and ease of massive parallel sequencing on various platforms and long-read sequencing with the MinIon (Oxford Nanopore) or PacBio (Menlo Park, CA), whole genomes of suspected biothreat agents can be quickly and easily obtained. However, the sequence differences may be limited to only informative single-nucleotide sequence (SNP) differences as represented by the genus *Bacillus* (Derzelle et al., 2015). Genetically similar microbes are analyzed via comparative genomics tools where multiple SNP patterns (canonical SNPs (canSNPs)) emerge and can distinguish geographically unique strains from one another when compared with reference strains of known biothreat agents (Dahiya, 2017). Many different phylogenetic tools and software are available now to perform such genetic comparisons. Working groups such as the AniBio Threat project (anibiothreat) in Europe developed standard protocols and expanded molecular databases to better assist in determining if an outbreak is natural or intentional (Derzelle and Thierry, 2013).

Both scientists and legal analysts need to understand (or at least appreciate) the results and their significance. Moreover, the degree of confidence that can be placed on a result must be understood so that the weight of a comparative analysis is not overstated. Basic statistics, probability, and population genetics are essential requirements of any curriculum involving the analysis of forensic biological evidence.

Bioinformatics

The term “bioinformatics” was developed as a result of the Human Genome Project. Because of the immense amount of data generated, it became necessary to apply more sophisticated computational techniques beyond what the average bench biologist had available. Bioinformatics requires a

combination of data handling and analysis skills (including standard statistics) that connect routine biology with high-powered computation. As scientific investigations and data generation expand using high resolution, deep sequencing of genomes of microbes, and large-scale proteomics, computational analyses will be more critical than ever. This subject can be taught in a simplistic form for the biologist or a more complex form for the computationally inclined scientist.

This interdisciplinary field has greatly impacted microbiology as well as forensics, medicine, agriculture, and other disciplines. Bioinformatic algorithms have simplified comparative genomics. Bioinformatics for medicine, microbial genome, and agriculture (Dahiya, 2017) are able to determine biologically relevant patterns in complex datasets (Damaso et al., 2018) as well as allow analyses of whole-genome shotgun sequencing of complex mixtures such as microbiomes (Citation: Chen and Pachter, 2005). The information derived from complex data will have to be extracted using algorithms such as support vector machines (Xu et al., 2015; Schlecht et al., 2008), neural networks (Vidaki et al., 2017; Vidaki and Kayser, 2017), and other more complex algorithms yet to be developed. All scientists and individuals with interest in microbial forensic sciences will need to have a basic understanding of statistics and bioinformatic tools.

Indicators of engineering

With rapid developments in molecular biology to benefit humankind also comes a great potential for manipulating a microorganism for nefarious purposes. Microbes could be engineered to be more virulent, and difficult-to-obtain microbes may be synthesized de novo in a laboratory. There is a need to detect not only the microbe but to determine if it was genetically manipulated or perhaps is a novel chimera.

Signatures indicative of manipulations or synthesis may be detected through sequencing and bioinformatic analyses. The skills and materials needed to manipulate a microbial genome may provide clues about the perpetrator and degree of sophistication used to develop the biothreat agent. This capability should be of interest to law enforcement and the intelligence community for supporting investigative leads.

Population genetics

A knowledge of population genetics is essential for understanding the rarity of a genetic (and sometimes protein) profile derived from an evidence sample. Molecular epidemiology is increasingly applying the principles of evolutionary and population genetics to pathogens. It is important to understand what constitutes a sample population as opposed to a sample collection, the mode of inheritance related to a genetic marker, what significance or weight to apply to a genetic marker, what the mutation rate of a marker is, and how to combine the weight of multiple markers. Training of the student in this discipline will require basic genetic courses and more advanced courses in phylogenetic analyses and other forms of comparison. Such educational material will be found in population genetics and systematic and evolutionary biology programs. The population genetics of pathogens and its importance for microbial forensics are covered elsewhere in this book.

An example of a forensic application of population genetics is the human skin microbiome. Schmedes et al. assessed human skin microbiome populations and, using supervised learning algorithms, were able to associate specific human microbiome profiles with their hosts with a high degree of accuracy (Schmedes et al., 2017). Recent comprehensive skin microbiome analyses (Ross et al., 2018) suggests that the skin microbiota have undergone coevolution with their corresponding mammalian hosts

providing support for future development of forensic applications using skin microbiomes (Ross et al., 2018).

Nonbiological tools

This topic is broad and can encompass tools that characterize a microbe morphologically or chemically. Subjects will range from microscopy to basic chemistry to analytical chemistry. The Amerithrax investigation demonstrated the importance of nonbiological measurements on samples of biological agents. A variety of mass spectral, spectroscopic, and other instrumental methods were used in an attempt to answer questions related to how, when, and what materials were used to produce the spore powders. Such information can be used to compare evidence directly to a reference sample or indirectly to infer something about the processes used to culture, stabilize, and/or disseminate the biothreat agent.

Bioelectronic scent detectors have also been developed using human olfactory and taste receptors that detect microbes in drinking water (Son et al., 2015; MankiSona et al., 2017). These human receptors have been shown to be capable of distinguishing one trillion different olfactory targets (Bushdid et al., 2014). Dogs have also been used for the detection of scents to track human waste in microbial source tracking in storm drains (Canine Scent Detection and Microbial Source Tracking of Human Waste Contamination in Storm Drains, 2014).

Forensic science

Forensic science is the application of science to answer questions of interest to a legal system as well as for military or state decisions (1,5,6). While science may not offer definitive solutions to the problems of society, it does serve a special investigative role, particularly in the criminal

justice system. The areas of science that have been traditionally exploited are diverse, but typically include the major disciplines of biology, chemistry, physics, and geology. Within each discipline are many scientific subcategories that may be used in a forensic science investigation. For example, within the discipline of biology are the subdisciplines of medicine, pathology, molecular biology, immunology, odontology, serology, psychology, and entomology.

The specific discipline(s) employed depends on the circumstances of the crime. Mathematics, especially statistics, is used to place weight or significance on observations or data retrieved from crime scene evidence. The ultimate question addressed by forensic science is usually “who committed the crime?” (i.e., attribution) or “who did not commit the crime?”, and crime scene evidence can play a role in answering these questions. Evidence can be any material, physical or electronic, that can associate or exclude individuals, victim, and/or suspect with a crime. It typically comprises materials specific to the crime as well as control samples for background information. Types of evidence may be fingerprints, blood, semen, saliva, hair, fibers, documents, photos, computer files, videos, firearms, glass, metals, plastics, paint, powders, explosives, tool marks, and soil. Scientists and other practitioners need to be cognizant of the types of evidence, how these different forms of evidence interplay, and how they can be used to help reconstruct the crime and/or identify the perpetrator.

Case histories

A case history is a detailed account of a person or event. Studies of case histories are instructive because they provide analysis of information in the relevant context, including real complexities. The study of a variety of incidents can be tailored to the particular group learning about them (see Chapters 1, 3, 10, 17 and 39). The Amerithrax case is likely to be studied for years by many

different groups ranging from scientists to law enforcement to lawyers. In addition to this case, many other cases are described in chapters of this book and the previous edition (Budowle et al., 2005), as well as in specific publications [(Schutzer et al., 2005) (Seth Carus, 1998)]. Among these threats are foodborne illnesses from bacteria, such as *Shigella* and *Salmonella*, and toxins such as ricin. In addition, there have been events involving agriculture, including a foot-and-mouth outbreaks in the United Kingdom (Jamal and Belsham, 2013) and mad cow disease in the United States (Sigurdson et al., 2018). Environmental contamination is also an area of interest (Yates et al., 2016; Cano and Toranzos, 2018), such as microbial source tracking to detect water contamination by human sewage (see Chapters 6 and 7) or animal farming such as by the poultry industry in Arkansas. Perhaps the most common area where issues of natural versus intentional events arise is related to emerging infections. This question has arisen with the outbreaks of influenza H1N1, severe acute respiratory syndrome, monkeypox, and specific cases of HIV infection (Jester et al., 2018; Lucas and Nelson, 2015; Morand et al., 2017).

Legal issues

Legal issues are of obvious importance to the legal community but are also important to the scientific community. There will be times when the evidence will be used in a court of law to prosecute an individual who has been arrested for a biocrime. There are standards for admissibility of scientific evidence in a legal setting. The scientist may be asked to provide expert testimony. These standards need to be known and appreciated so that admissibility of evidence can be achieved. The government will use microbial forensic scientists and other experts, their results, the scientific literature, and supporting validation studies to support its position. The

defense will defend its client vigorously to attempt to achieve an acquittal. Because of the adversary system, challenges are expected to the credibility of the science and its practitioners in the United States and other English-based law countries (Harmon et al., 2005; Kirsch and Daubert, 1995). Studying the science behind headlines can be a very instructive and creative way to interest students. Some controversial issues in forensic DNA, which can be used instructively, involve (1) the use of low quantities of template DNA (Budowle et al., 2009; Gilder et al., 2009), (2) population genetics issues and associated degrees of confidence (NRC), (3) potential contamination and background signals, (4) sufficient and appropriate validation studies, and (5) access to proprietary information or intellectual property.

The standards and court proceedings, however, will vary for each country. For example, in the United States, possession of unauthorized material can be considered a crime by itself. Therefore, an understanding of the relevant laws for handling and possessing, for example, select agents is important.

Operational and intelligence issues

Evidence derived from a microbial forensic investigation may not necessarily end up in court. For example, such evidence can be used for intelligence purposes. Information can be gathered to determine the risk or probability of an individual, a group, or a state to use (or has used) a bioweapon in an attack. The primary goal is to intercede and thwart the attack before it can happen. Alternatively, if an attack has occurred, a head of state may require some evidence to determine whether to retaliate and to whom retaliation should be directed. Results from microbial evidence are far reaching and have consequences. Training individuals in understanding the strengths and limitations of scientific evidence is essential so that proper decisions and responses can be made. Understanding how information is gathered, analyzed, and

acted upon is likely to be of interest to any level of student.

National-level capabilities and resources

Policy and decision makers need to learn about and support advances in microbial forensic strategies and capabilities, such as were described in the "National Science and Technology Council, National Research and Development Strategy for Microbial Forensics, Office of Science and Technology Policy (2009) (National Science and Technology Council, 2009), and Science Needs for Microbial Forensics: Developing Initial International Research Priorities, 2014 (Science Needs for Microbial Forensics, 2014)." The following aspects should be addressed: (i) What and how should a country be prepared? (ii) What strategies make sense? (iii) Planning, implementing, and measuring effectiveness. (iv) Training and evaluation exercises. (v) Where can additional support be sought? (vi) Leveraging of epidemiological tools.

Conclusion

Education of the next generation of microbial forensic scientists, the continuing professional development and training of practitioners, and informing those in the legal community and policy makers are our collective responsibility and of paramount importance. The evolution of technology, analytical capabilities, and, equally as important, the need for incorporating advances into our education and training programs continue to evolve and expand rapidly. This chapter along with the educational resources cited indicates some resources that could be considered to educate and train those interested in the field of microbial forensics. Other forms of education should include didactic lectures, workshops, conferences, practical demonstrations, and discussions at specialty meetings. Those interested in the development of formal microbial forensic degree programs will find

resources from academic, government, industry, and professional societies. The target audience may include laboratory directors, bench scientists, other practitioners, faculty, college students, law enforcement representatives, medical care and first responder personnel, lawyers, and judges. Those who fulfill teaching roles, whether by profession or indirectly as reporters and even entertainment writers, can become informed so that their writings are founded in facts that could serve as well to better educate stakeholders.

References

- American Association for the Advancement of Science (AAAS), 2011. Vision and Change in Undergraduate Biology Education: A Call to Action. American Association for the Advancement, Washington, D.C, 6. 2010-2013; <http://www.anibiothreat.com/>).
- Budowle, B., Beaudry, J.A., Barnaby, N.G., Giusti, A.M., Bannan, J.D., Keim, P., August 2007. Role of law enforcement response and microbial forensics in investigation of bioterrorism. *Croat. Med. J.* 48 (4), 437–449.
- Budowle, B., Eisenberg, A.J., van, D.A., 2009. Validity of low copy number typing and applications to forensic science. *Croat. Med. J.* 50, 207–217.
- Budowle, B., Schmedes, S.E., Wendt, F.R., 2017. Increasing the reach of forensic genetics with massively parallel sequencing. *Forensic Sci. Med. Pathol.* 13 (3), 342–349. <https://doi.org/10.1007/s12024-017-9882-5>. Epub 2017 Jun 19.
- Budowle, B., Schutzer, S.E., Ascher, M.S., Atlas, R.M., Burans, J.P., Chakraborty, R., Dunn, J.J., Fraser, C.M., Franz, D.R., Leighton, T.J., Morse, S.A., Murch, R.S., Ravel, J., Rock, D.L., Slezak, T.R., Velsko, S.P., Walsh, A.C., Walters, R.A., 2005. Towards a system of microbial forensics: from sample collection to interpretation of evidence. *Appl. Environ. Microbiol.* 71 (5), 2209–2213.
- Budowle, B., Schutzer, S., Breeze, R., Keim, P., Morse, S. (Eds.), 2005. *Microbial Forensics*. Academic Press, San Diego.
- Budowle, B., Schutzer, S.E., Burans, J.P., Beecher, D.J., Cebula, T.A., Chakraborty, R., Cobb, W.T., Fletcher, J., Hale, M.L., Harris, R.B., Heitkamp, M.A., Keller, F.P., Kuske, C., LeClerc, J.E., Marrone, B.L., McKenna, T.S., Morse, S.A., Rodriguez, L.L., Valentine, N.B., Yadev, J., 2006. Quality sample collection, handling, and preservation for an effective microbial forensics program. *Appl. Environ. Microbiol.* 72 (10), 6431–6438.
- Budowle, Schutzer, S.E., Einseln, A., Kelley, L.C., Walsh, A.C., Smith, J.A., et al., 2003. Public health: building microbial forensics as a response to bioterrorism. *Science* 301, 1852–1853.
- Bushdid, C., Magnasco, M.O., Vossball, L.B., Keller, A., March 21, 2014. Humans can discriminate more than 1 trillion olfactory stimuli. *Science* 343 (6177), 1370–1372. <https://doi.org/10.1126/science.1249168>.
- Cano, R.J., Toranzos, G.A. (Eds.), 2018. *Environmental Microbial Forensics*. Publication Year. <https://doi.org/10.1128/9781555818852>. Print ISBN : 9781555815042e-ISBN : 9781555818852.
- Carter, D.O., Tomberlin, J.K., Benbow, M.E., Metcalf, J.L., 2017. *Forensic Microbiology*. John Wiley & Sons Ltd. <https://doi.org/10.1002/9781119062585> © 2017. Print ISBN:9781119062554 |Online ISBN:9781119062585.
- Christopher, A., Bidwell, J.D., Bhatt, K., February 2016. Use of Attribution and Forensic Science in Addressing Biological Weapon Threats: A Multi-Faceted Study A Special Report Published by the Federation of American Scientists. <https://fas.org/wp-content/uploads/2016/03/bioattribution-nps-report-3-14.pdf>.
- Citation: Chen, K., Pachter, L., 2005. Bioinformatics for whole-genome shotgun sequencing of microbial communities. *PLoS Comput. Biol.* 1 (2), e24. <https://doi.org/10.1371/journal.pcbi.001002>.
- Dahiya, B.P., 2017, 1938-194 J. *Pharmacol. Phytochem.* 6. are able to determine biologically relevant patterns in complex data sets (add my paper here).
- Damaso, N., Mendel, J., Mendoza, M., von Wettberg, E.J., Narasimhan, G., Mills, D., July 2018. Bioinformatics approach to assess the biogeographical patterns of soil communities: the utility for soil provenance. *J. Forensic Sci.* 63 (4), 1033–1042. <https://doi.org/10.1111/1556-4029.13741>. Epub 2018 Jan 22.
- Derzelle, S., Girault, G., Kokotovic, B., Angen, Ø., 2015. Whole genome-sequencing and phylogenetic analysis of a historical collection of *Bacillus anthracis* strains from Danish Cattle. *PLoS One* 10 (8).
- Derzelle, S., Thierry, S., September 2013. Biosecurity and bioterrorism: biodefense strategy, practice, and science, 11 (S1). August 24, 2013.
- Emmert, E.A.B., the ASM Task Committee on Laboratory Biosafety, May 2013. Biosafety guidelines for handling microorganisms in the teaching laboratory: development and Rationale. *J. Microbiol. Biol. Educ.* 14, 78–83. <https://doi.org/10.1128/jmbe.v14i1.531>.
- Eybpoosh, S., Haghdoost, A.A., Mostafavi, E., Bahrapour, A., Kayhan Azadmanesh, Zolala, F., August 2017. *Electron physician*, 9 (8), 5149–5158. <https://doi.org/10.19082/5149> PMID: PMC5614305 PMID: 28979755. Published online 2017 Aug 1.

- Gil, C., Monteoliva, L., January 31, 2014. Trends in microbial proteomics. *J. Proteomics* 97, 1–2. <https://doi.org/10.1016/j.jprot.2013.12.015>.
- Gilder, Koppl, R., Kornfield, I., Krane, D., Mueller, L., Thompson, W., 2009. Comments on the review of low copy number testing. *Int. J. Leg. Med.* 123, 535–536.
- Harmon, R., 2005. Admissibility standards for scientific evidence. In: Breeze, R.G., Budowle, B., Schutzer, S.E. (Eds.), *Microbial Forensics*. Academic Press, San Diego, pp. 381–392.
- Horak, R.E.A., Merkel, S., Chang, A., May 2015. The asm curriculum guidelines for undergraduate microbiology: a case study of the advocacy role of societies in reform efforts. *J. Microbiol. Biol. Educ.* 16 (1), 100–104. <https://doi.org/10.1128/jmbe.v16i1.915>. Published online 2015 May 1.
- <https://www.asm.org/index.php/asm-meetings>.
- <https://www.aafs.org/home-page/meetings/international-educational-outreach-program/>.
- <https://www.asm.org/index.php/education>.
- <https://fas.org/wp-content/uploads/2016/03/bioattribution-nps-report-3-14.pdf>.
- <http://www.cofse.org/>.
- <https://www.un.org/disarmament/wmd/bio/>.
- http://www.edsource.org/pub_mathscience1-08_report.html.
- <https://emergency.cdc.gov/bioterrorism/prep.asp>.
- <http://www.fepac-edu.org/>.
- <https://www.aafs.org/home-page/meetings/forensic-science-educational-conferences/>.
- <https://www.frontiersin.org/articles/10.3389/fbioe.2015.00080/full>.
- <http://www.ncstl.org/>.
- <http://www.nsta.org/publications/news/story.aspx?id=59152>.
- <https://www.nfstc.org/>.
- <https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science/interdisciplinary-activities>.
- <https://www.phe.gov/Preparedness/responders/ndms/ndms-teams/Pages/dmort.asp>.
- <http://www.project2061.org/>.
- <https://www.publichealthonline.org/epidemiology/masters-degree-programs/https://www.usnews.com/education/best-global-universities/slideshows/see-the-top-10-global-universities-for-molecular-biology-and-genetics> <https://www.usnews.com/education/best-global-universities/microbiology> <https://www.usnews.com/best-graduate-schools/top-science-schools/microbiology-rankings>.
- <https://www.asm.org/index.php/scientific-program-ngs-2018/ngs-program-by-day>.
- <https://www.aafs.org/home-page/meetings/>.
- <http://visionandchange.org/abstract/a-new-microbiology-curriculum-based-on-vision-change/>.
- <https://yfsf.aafs.org/>.
- Jagielski, T., Minias, A., van Ingen, J., Rastogi, N., Brzostek, A., Żaczek, A., JarosławDziadek, February 2016. Methodological and clinical aspects of the molecular epidemiology of *Mycobacterium tuberculosis* and other mycobacteria. *Clin. Microbiol. Rev.* 29 (2), 239–290. <https://doi.org/10.1128/CMR.00055-15>.
- Jamal, Belsham, 2013. Foot-and-mouth disease: past, present and future. *Vet. Res.* 44, 116.
- Jester, B., Uyeki, T.M., Jernigan, D.B., Tumpey, T.M., November 16, 2018. Historical and clinical aspects of the 1918 H1N1 pandemic in the United States. *Virology* 527, 32–37. <https://doi.org/10.1016/j.virol.2018.10.019> ([Epub ahead of print]).
- Canine scent detection and microbial source tracking of human waste contamination in storm drains. *Water Environ. Res.* 86 (6), June 2014. <https://doi.org/10.2175/106143013X13807328848496>.
- Karlsson, O.E., Hansen, T., Knutsson, R., Löfström, C., Granberg, F., Berg, M., 2013. Metagenomic detection methods in biopreparedness outbreak scenarios. *Biosecur. Bioterror. Biodefense Strategy Pract. Sci.* 11. No. S1DetectionFree AccessOpen Access license.
- Kirsch, E.W., Daubert, v., 1995. Merrell Dow Pharmaceuticals: active judicial scrutiny of scientific evidence. *Food Drug Law J.* 50, 213–234.
- Lucas, S., Nelson, A.M., January 2015. HIV and the spectrum of human disease. *J. Pathol.* 235 (2), 229–241. <https://doi.org/10.1002/path.4449>.
- MankiSona, D.K., HwijinKo, S.H., Parka, T.H., January 15 , 2017. A portable and multiplexed bioelectronic sensor using human olfactory and taste receptors. *Biosens. Bioelectron.* 87, 901–907. <https://doi.org/10.1016/j.bios.2016.09.040>.
- McKay, L.N., ASM MINAH Undergraduate Curriculum Guidelines Committee, April 27, 2018. Microbiology in nursing and allied health (MINAH) undergraduate curriculum guidelines: a call to retain microbiology lecture and laboratory courses in nursing and allied health programs. *J. Microbiol. Biol. Educ.* 19 (1) <https://doi.org/10.1128/jmbe.v19i1.1524>. eCollection 2018 pii: 19.1.51.
- Merkel, S.M.1, August 2016. American Society for Microbiology resources in support of an evidence-based approach to teaching microbiology. pii: fnw172 FEMS Microbiol. Lett. 363 (16). <https://doi.org/10.1093/femle/fnw172>. Epub 2016 Jul 12.
- Merkel, S., the ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology, 2012. The development of curricular guidelines for introductory microbiology that focus on understanding, 2012 *J. Microbiol. Biol. Educ.* 13 (1), 32–38. Published online 2012 May 3.
- Morand, A., Delaigue, S., Morand, J.J., February 1, 2017. Re-view of poxvirus: emergence of monkeypox. *Med. Sante Trop.* 27 (1), 29–39. <https://doi.org/10.1684/mst.2017.0653>.

- National Science, Technology Council, 2009. National Research and Development Strategy for Microbial Forensics. Office of Science and Technology Policy.
- Nolte, K., 2003. Homeland security and emergency preparedness- the potential role of medical examiners and coroners in responding to and planning for bioterrorism and emerging infectious diseases. In: Institute of Medicine (US) Committee for the Workshop on the Medicolegal Death Investigation System. National Academies Press (US), Washington (DC) (Chapter 9).
- NRC, 2011. Successful K-12 STEM Education: Identifying Effective Approaches in Science, Technology, Engineering, and Mathematics. <https://www.nap.edu/catalog/13158/successful-k-12-stem-education-identifying-effective-approaches-in-science>.
- NRC 2 1998.
- Riley, L.W., 2004. Molecular Epidemiology of Infectious Diseases: Principles and Practices. ASM Press, Washington, DC (Add references:).
- Ross, A.A., Müller, K.M., Weese, J.S., Neufeld, J.D., 2018. Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phyllosymbiosis within the class Mammalia. PNAS. Published ahead of print June 5, 2018. <https://doi.org/10.1073/pnas.1801302115>.
- Schlecht, J., Kaplan, M.E., Barnard, K., Karafet, T., Hammer, M.F., Merchant, N.C., June 2008. Machine-learning approaches for classifying haplogroup from Y chromosome STR data. PLoS Comput. Biol. 4 (6), e1000093. <https://doi.org/10.1371/journal.pcbi.1000093>. Published online 2008 Jun 13.
- Schmedes, S.E., Sajantila, A., Budowle, B., August 2016. Expansion of microbial forensics. J. Clin. Microbiol. 54 (8), 1964–1974. <https://doi.org/10.1128/JCM.00046-16>.
- Schmedes, S.E., Woerner, A.E., Budowle, B., 2017. Forensic human identification using skin microbiomes. Appl. Environ. Microbiol. 83 e01672-17. <https://doi.org/10.1128/AEM-01672-17>.
- Schutzer, S.E., Budowle, B., Atlas, R.M., December 2005. Biocrimes, microbial forensics, and the physician. Published online 2005 Sep. 27 PLoS Med. 2 (12), e337. <https://doi.org/10.1371/journal.pmed.0020337>. PMID: PMC1236212 PMID: 16167845.
- Science Needs for Microbial Forensics, 2014. Developing Initial International Research Priorities. <http://dels.nas.edu/Report/Science-Needs-Microbial-Forensics-Developing/18737>.
- Seth Carus, W., August 1998. Bioterrorism and Biocrimes the Illicit Use of Biological Agents since 1900. February 2001 Revision. Center for Counterproliferation Research
- National Defense University Washington, D.C. ISBN-13: 978-1410100238 ISBN-10: 1410100235.
- Sigurdson, C.J., Bartz, J.C., Glatzel, M., October 2018. Cellular and molecular mechanisms of prion disease. Annu. Rev. Pathol. <https://doi.org/10.1146/annurev-pathmechdis-012418-013109>.
- Son, M., Cho, D.-guk, Lim, J.H., Park, J., Hong, S., Koe, H.J., Park, T.H., December 15 , 2015. Real-time monitoring of geosmin and 2-methylisoborneol, representative odor compounds in water pollution using bioelectronic nose with human-like performance. Biosens. Bioelectron. 74, 199–206.
- Teshome, B., 2016. Microbes as biological weapons-a review Befekadu Teshome. Int. J. Mod. Chem. Appl. Sci. 3 (1), 323–325. Page No.323.
- Tomkovich, S., Jobin, C., January 2016. Microbiota and host immune responses: a love–hate relationship. Published online 2015 Nov 2 Immunology 147 (1), 1–10. <https://doi.org/10.1111/imm.12538>. PMID: PMC4693877. PMID: 26439191.
- Biological Security The Countering Weapons of Mass Destruction (CWMD) Office was Established in December 2017 by Consolidating Primarily the Domestic Nuclear Detection Office, A Majority of the Office of Health Affairs, As Well As Other DHS Elements <https://www.dhs.gov/topic/biological-security>.
- United States, Federal Bureau of Investigation, Laboratory FBI, 1999. Handbook of Forensic Ser Vices. Available from: <http://www.fbi.gov/hq/lab/handbook/intro.htm>.
- Vidaki, A., Ballard, D., Aliferi, A., Miller, T.H., Barron, L.P., May 2017. Denise Syndercombe Court. (DNA methylation-based forensic age prediction using artificial neural networks and next generation sequencing. Forensic Sci. Int. Genet. 28, 225–236. <https://doi.org/10.1016/j.fsigen.2017.02.009>.
- Vidaki, A., Kayser, M., 2017. From forensic epigenetics to forensic epigenomics: broadening DNA investigative intelligence. Genome Biol. 18, 238. <https://doi.org/10.1186/s13059-017-1373-1>. Published online 2017 Dec 21.
- Xu, C., Qu, H., Wang, G., Xie, B., Shi, Y., Yang, Y., Zhao, Z., Hu, L., Fang, X., Yan, J., Lei, 2015. A novel strategy for forensic age prediction by DNA methylation and support vector regression model. FengSci. Rep. 5, 17788. <https://doi.org/10.1038/srep17788>. Published online 2015 Dec 4.
- Yates, M.V., Nakatsu, C.H., Miller, R.V., Pillai, S.D. (Eds.), 2016. Manual of Environmental Microbiology, fourth ed. <https://doi.org/10.1128/9781555818821> Print ISBN : 9781555816025, e-ISBN : 9781555818821. www.aafs.org.