

Science Supporting Attribution in the BWC Context

Randall S. Murch, PhD
Virginia Tech University, USA
BWC Experts Group, Geneva CH
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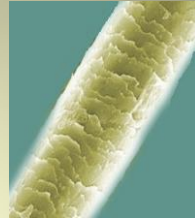
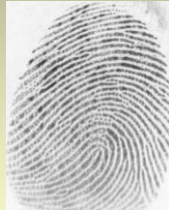
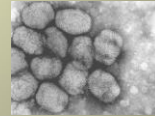
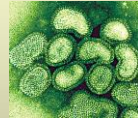


Assumptions

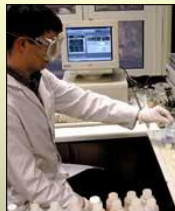
- If the BWC were to adopt an aggressive, yet measured, approach to investigating suspicious or anomalous disease outbreaks or clearly indicated illicit use of disease agents and biotoxins
 - robust science, which should be expected to be accurate, reliable, repeatable and defensible, would be an important contributor to informing investigative, legal and policy decisions and follow on actions
 - a supporting global scientific enterprise should be available on demand, resourced and held accountable to:
 - meet high expectations for responsiveness to stakeholder requirements at all levels
 - be applied to a dynamic range of operational and investigative situations
 - be based on sound science and practice which provides significant value
 - be standardized for quality, credibility, transparency and defensibility,
 - and evolve matched to
 - the availability and leverage of new science, technology and innovation
 - address prioritized gaps to substantially inform crucial investigative, legal and policy questions
 - develop needed capabilities against known and future threats including the misuse of associated science and technology
- A harmonized global scientific resource to support the BWC does not now exist, but could with a concerted, organized and resourced campaign with recognized, expert enterprise and SME leadership

Potential Scenarios to Frame the Microbial Forensics Discussion

- High Impact Bio Attack (Small ↔ Large), No Warning
- High Impact Attack (Small ↔ Large), Warning
- Broad or Surgical Strike, Military vs. Civilian Targets (Personnel, Infrastructure, System)
- Suspicious or Anomalous Disease Outbreak
- Suspicious or Anomalous Event Claimed to be Accidental
- Intelligence, Investigation Suggesting Imminent Attack
- Possible Attack or Claim, Hoax



Introduction to Forensic Science



Forensic Science

- Application of science in the investigation of legal matters (criminal and civil)...and now intelligence, operations and policy
- Scientific knowledge and technology are used to serve as independent witnesses
- Science may not offer definitive solutions for all scenarios; it does provide a special investigative role
- Goal is “attribution” – i.e., who committed the crime/is responsible for the act or event

“Forensics” Integrates Science with the Investigative Process

- Biology
- Chemistry
- Materials
- Trace Evidence
- Impression and Pattern
- Engineering
- Digital-Computer
- Reconstructive
- Medicine & Pathology



Terms of Reference

- **Forensic Science:** Analysis and interpretation of physical evidence to determine its relevance to events, people, places, tools, methods, processes, intentions, plans
 - *Identification and Characterization (What is it? Who is it? Does it add value to the investigation?)*
 - *Inclusion toward Attribution or Exclusion (Where did it come from? How does it relate to something, some place or someone of interest and how?)*
- **Scientific Attribution:** Assignment of a ***sample of questioned origin*** to ***a source of known origin*** to a **high degree of scientific certainty** (at the same time excluding origination from other sources)
 - Attribution requires comparison of samples from “Q” (Questioned) and “K” (Known) sources
 - Quantitative and qualitative expression based on what analysis can provide and how it can be communicated
 - Increasingly and more intensely, the science underlying “attribution” is being scrutinized, expectations are increasing for accuracy, reliability, validity
- **Legal/Policy Attribution: “Who Did It and Who is Involved?”** (“beyond a reasonable [or acceptable level of] doubt”)

Some Key Objectives When Applying Forensic Science

- Identify and provide useful (“probative”) information from physical evidence that aids investigation, prosecution or exoneration
- Provide leads; helps to inform and guide investigations and investigative questions, prosecutive or defense theories and processes
- Help with “rule in-rule out” during investigations or similar
- Narrow or limit the number of sources that evidence could have originated from; ideally one source to definable/acceptable certainty
- Inform attribution (who did it or is involved?) and exoneration (who did not or could not have done it?) decisions
- Help to support, modify or discount prosecutive or defense theories and strategies before, during (and sometimes after) trial

Phases of Forensic Investigations

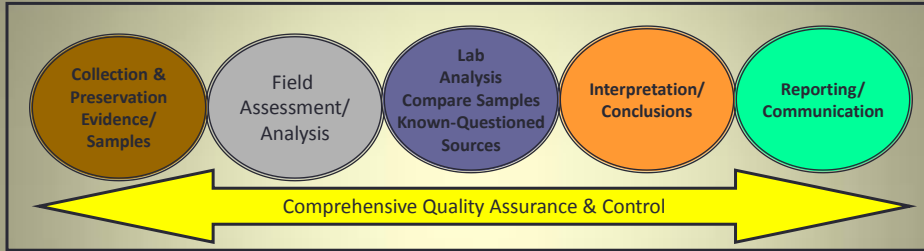
- **Intelligence & Information Gathering**
 - Based on Triggering Event or Information
 - Seek to Lay Foundation for and Justify Investigation, (Intelligence Activities)
- **Field Investigation**
 - Multidisciplinary, Multi-Source and Time-Driven
 - Rule In and Rule Out Process, Buttress or Re-Direct
- **Crime Scene Investigation**
 - Evidence Identification, Collection, Preservation,
 - Includes Presumptive Testing
- **Laboratory Analysis**
 - Includes Deeper Characterization and Intercomparison of Samples from “Questioned Sources” and “Known Sources”
- **Interpretation, Conclusions In Context**
 - Transition to Real-Time and End Users
 - Includes Alternative Interpretations
- **Building, Shaping Decisions and Actions**
 - Iterative, Aids Meeting “Burden of Proof”
 - Part of Build toward Prosecution, Can Support Exoneration
- **Communication and Decision Making**
 - To Legal & Policy Decision Systems, Other Stakeholders
 - Unbiased Rendering of Results, Conclusions, Explanations (including Alternative)



Goals for Forensic Methods

- Robust Collection & Preservation of Evidence
- Relevant Exploitation of Sample
- High Discrimination
- Enables Comparison of K and Q Samples
- Utility Across Known, Encountered Sample Types
- Accuracy
- Reliability
- Defined & Acceptable Error Rate
- Speed & Responsiveness
- Repeatability
- Transferability
- Validity Can Be Independently Established
- Results Probative, Interpretable, Explainable, Useable and Defensible

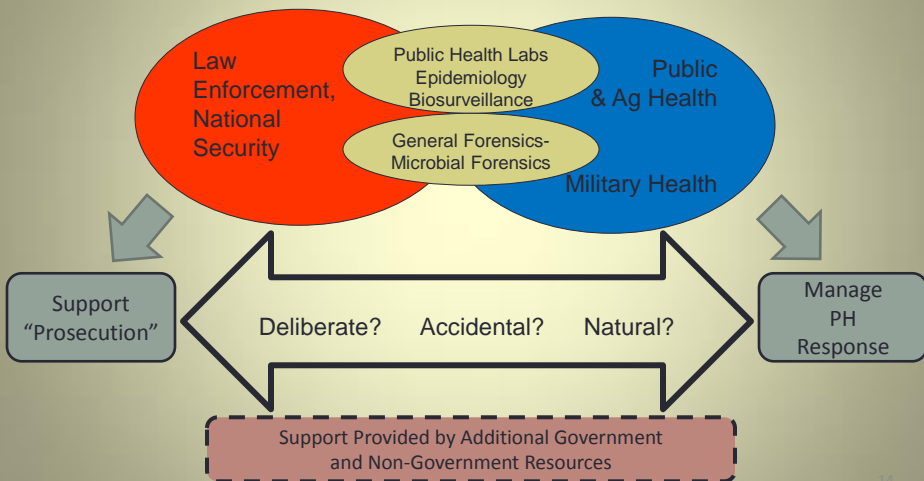
Ideal Forensic Science System



- Capabilities Matched to Submitter & Stakeholder Needs and Requests
- Appropriate Deployable Assets & Facilities with Sufficient Resources for Operation & Maintenance
 - Field, Transport & Laboratory Evidence Integrity & Security
 - Properly Credentialed, Trained and Certified Personnel
- Full Suite of Equipment, Fully Validated Methods: Matched to Samples Received & Questions Asked
 - Comprehensive Quality Assurance & Control Program
 - Appropriate Repositories and Data Bases
- Appropriate Resources: Research, Development, Validation, Technology Transfer

Special & Dynamic Collaboration is Required for Investigating Possible Bioattacks

(Pre-Event) Event Outset, During Event, Post-Event



Introduction to Microbial Forensics

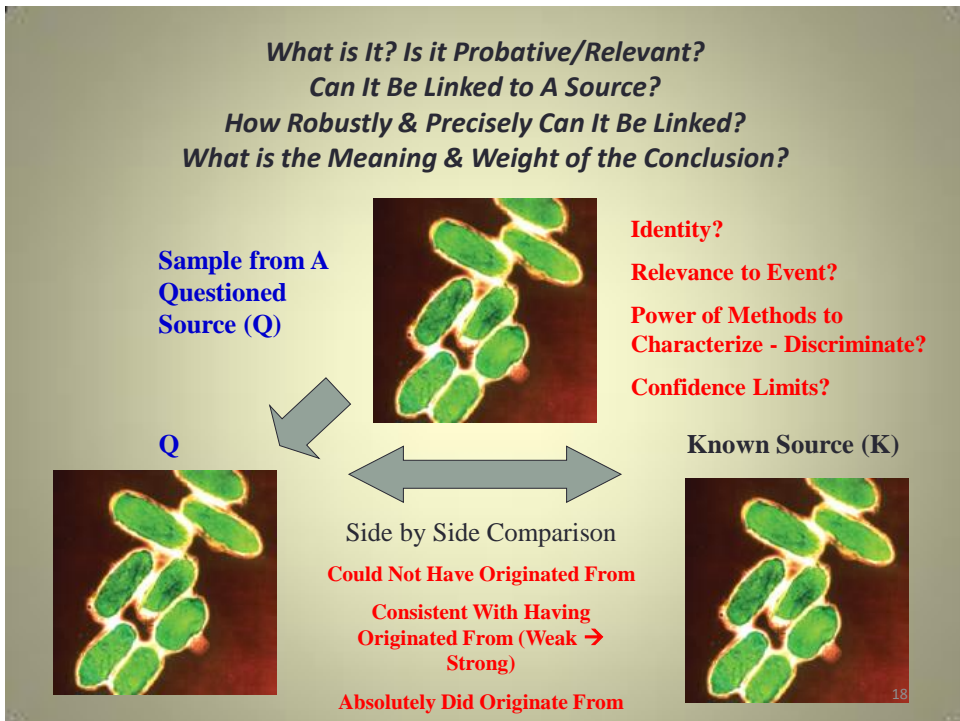
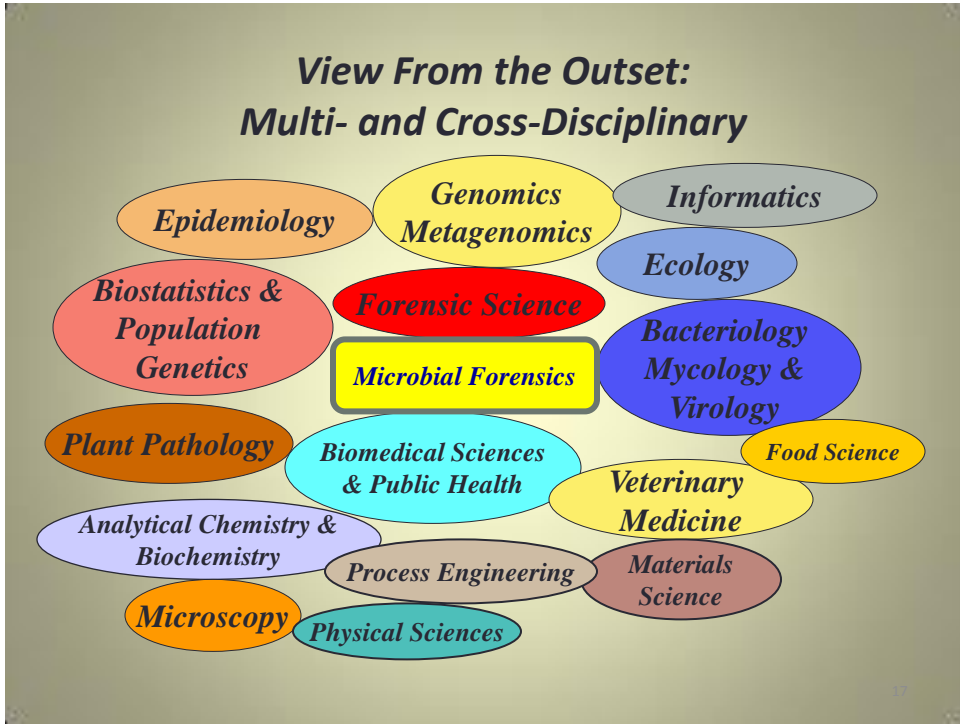
Application of Forensic Science and Related Sciences to Problems Involving Biological Threat Agents, Their By Products and Associated Physical Evidence



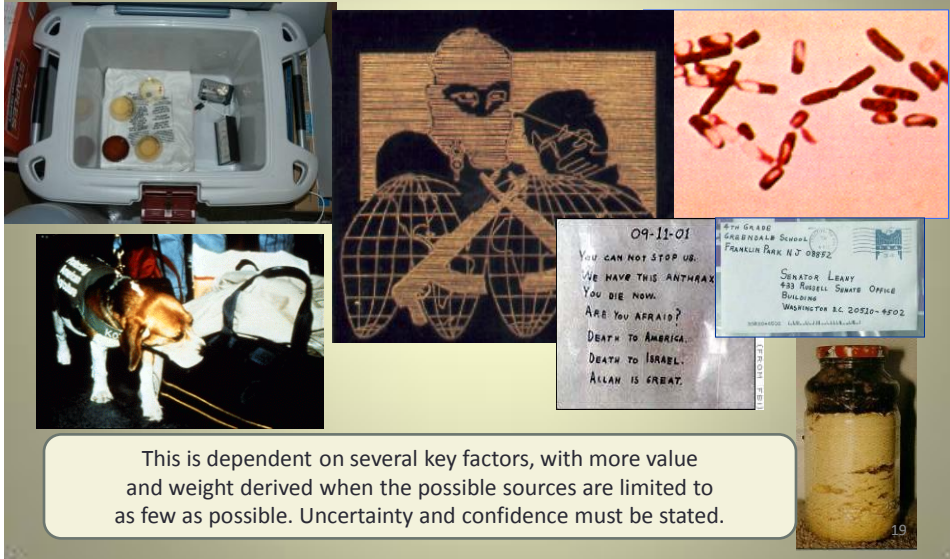
Collect, Preserve & Transport, Triage, Analyze Interpret, Integrate, Decide

Microbial Forensics (b. 1996): A Brief History

- Established in the U.S. as Capability to Support Bioterrorism Investigations
 - Provide Leads
 - Support Prosecutions, Exonerations
- Drew from Legacy Science, Many Fields
- Incorporated Forensic Science, Principles & Practices
- Sensitive to Legal Requirements
- Immediate Outreach to Public Health
 - Often Parallel Investigations, Similar Investigative Practices, Overlapping Questions to Address, Related Science and Technologies
- Early Thinking: Also Apply to Biowarfare and Bioproliferation, Not Just Law Enforcement/Bioterrorism
- Early Considerations: Apply at Onset, During and After Suspicious and Nefarious Activities and Events
- Tightly Couple Robust Science to Informing Key Investigative, Legal and Policy Questions



The Challenge for Any Forensic Analysis of a Bioweapon: Source Exclusion, Association and “Attribution”



Microbial Forensic Tools and Methods Provide Information to Determine “What”, “How”, “From Where”, Leading to “Who”

- What organism was used?
- What is the genetic sequence?
- What type of growth media was used?
- What special additives or equipment were used?
- What residual markers remain?
- What is the size and sophistication of the operation?
- Can we infer the processing methodology?
- What is the relationship of what was found in background (the environment) to what was found in the samples of interest?
- Can we correlate samples to specific source materials or to other samples?
- If the bioweapon was a toxin, what can be determined that can help inform the appropriate questions listed above?

Major Categories: Microbial Forensics Analyses & Outcomes

- Presumptive Testing
 - Primary Detection, Small Platforms, Rapid Results but High Error Rates and Limited Coverage
- Identification and Characterization
 - Confirmation, Identification, Low Error Rate, Greater Coverage, Larger Lab-Based Platforms
- Deep Analysis Toward Source Attribution
 - Genome-Based Analysis (Various Approaches), Highest Resolution through Whole Genome Sequencing and Powerful Bioinformatics, Precise Phylogenetic Placement (Strain/Isolate Level)
 - Complementary Analyses, Non-Genomic, Physical-Chemical
 - Classical Forensic Analyses, Including on Evidence Contaminated with Threat Agent/Hazard

CDC Bioterrorism Threat Agents

- Category A
 - Anthrax, *Bacillus anthracis*
 - Botulism, *Clostridium botulinum* toxin
 - Plague, *Yersinia pestis*
 - Smallpox, *Variola major*
 - Tularemia, *Francisella tularensis*
 - Viral Hemorrhagic Fever, Filoviruses (e.g., Ebola, Marburg) and Arenaviruses (e.g., Lassa, Machupo)
- Category B
 - Brucellosis, *Brucella* spp.
 - Epsilon Toxin, *C. perfringens*
 - Food Safety Threats (e.g., *Salmonella* spp., *Escherichia coli* O157:H7 et al, *Shigella* spp.)
 - Glanders (*Burkholderia mallei*)
 - Melioidosis, *B. pseudomallei*
 - Psittacosis (*Chlamydia psittaci*)
 - Ricin Toxin (Seeds from *Ricinus communis*)
 - *Staphylococcus enterotoxin B*
 - Typhus Fever (*Rickettsia prowazekii*)
 - Viral Encephalitis (e.g., Alphaviruses [VEE, EEE, WEE])
 - Water Safety Threats (e.g., *Vibrio cholerae* and *Cryptosporidium parvum*)
- Category C
 - Emerging Infectious Diseases (e.g., Nipah Virus and Hantavirus)

Are the forensic techniques needed in place today for all of these and can they meet the expectations of all levels of stakeholders? For all the others that could be used as bioweapons?

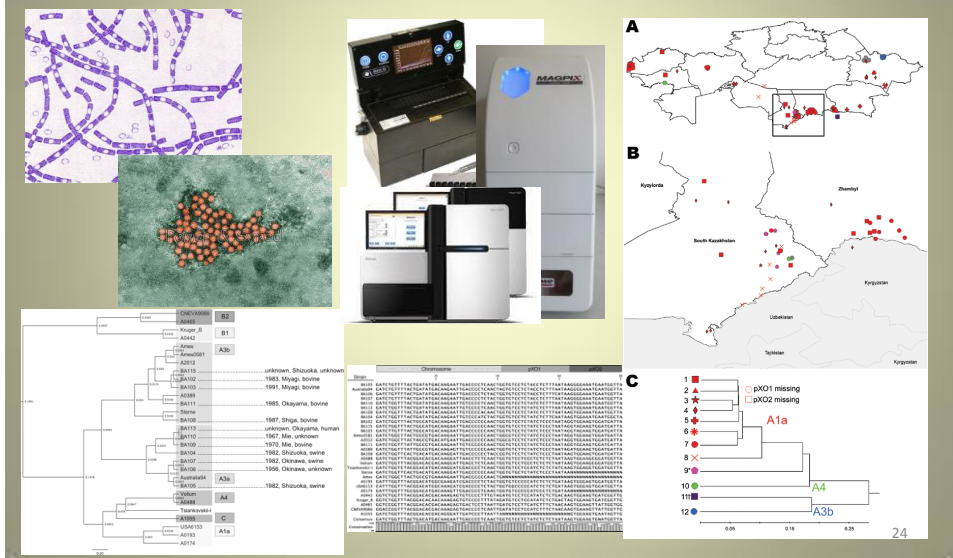
Magnitude of the Microbial Forensics Workspace

- Any infectious agent can be used as a biological weapon
- Over 1000 agents known to infect humans*
 - 217 virus species
 - 538 bacterial species
 - 307 fungi
 - 66 parasitic protozoa
- Numerous strain variations
- Emerging and re-emerging pathogens
- Potential bio-engineered organisms
- Pathogens of economically important plants and animals not counted (x 1000s)
- Biological toxins have been and can be used as weapons

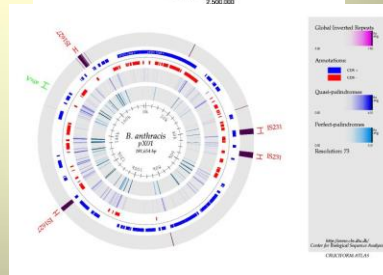
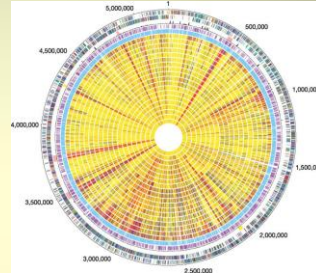
Some biological threat agents have been and are of greater interest than others as weapons, yet the development and use as weapons of any of the rest cannot be discounted.

*Taylor et al, *Phil. Trans. R. Soc. Lond. B* (2001) 356, 983-989

Basis for Biology-Based Analysis: Identification, Characterization, Phylogenetics, Phylogeography, and Features/Properties of Interest



Major Investment Areas: Sequencing Approaches and Informatics



Access to or Availability of
Appropriate Genomic Databases
For Comparisons is Crucial!!

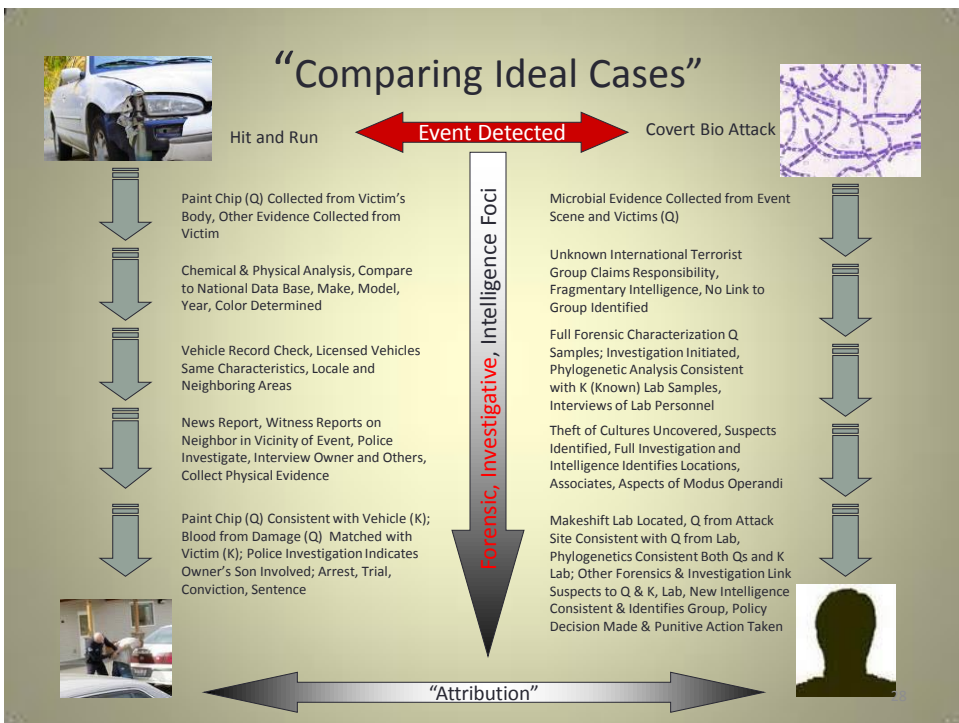
Challenge: What Can Be Learned from Weaponization Processes and Variations?

BW agents are generally mixtures that derive from a *matrix* of possible processing steps

Growth	Separation	Washing	Drying	Grinding	Additives
<ul style="list-style-type: none"> Carbon sources Nitrogen source Complex media Cell culture Animal hosts Agar 	<ul style="list-style-type: none"> Heat shock Filter Centrifuge Precipitation or flocculation Solvent partitioning Gradients 	<ul style="list-style-type: none"> Detergents Water/buffer Solvents e.g. FCs Spray-dry 	<ul style="list-style-type: none"> Air dry Azeotropic Acetone Spray dry Lyophilize 	<ul style="list-style-type: none"> Ball mill Jet mill Mortar & pestle 	<ul style="list-style-type: none"> Flow enhancers Resins Stabilizers Encapsulants Irritants

Of course, the chosen method may be very crude... or may not involve any "processing" at all

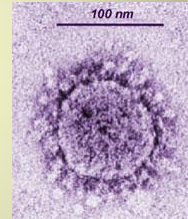
Forensic Analysis of Associated Traditional Evidence (including Contaminated)



Microbial Forensics:

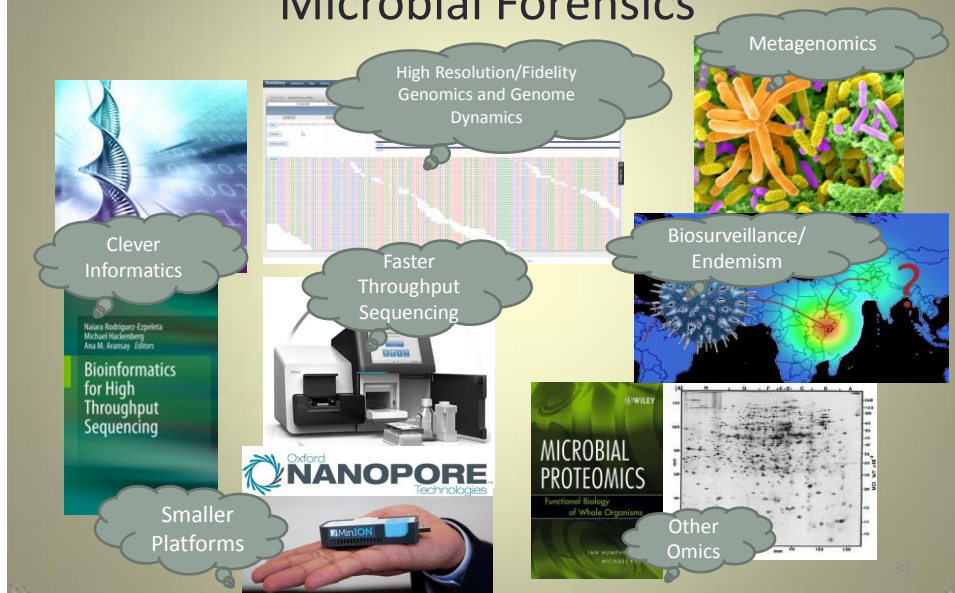
Emerging and Future Technologies, Some Are or May Be Here

- **Creative, Even Subtle Genetic Engineering**
- **Bioprospecting, Exploiting Natural Diversity**
- **Gene Shuffling & Directed Evolution**
- **Small Footprint Design & Production Systems**
- **Creative Denial and Deception**
- **Synthetic Biology**



Can "Passive" and "Active" Measures be Anticipated? Developed?

The Emerging Future for Microbial Forensics



Current State of Microbial Forensics

- Standing capability exists within the US Government in various departments and agencies; cooperative network exists which includes National Laboratories and academia ; science has advanced considerably since inception
- Science is drawn from other, closely related disciplines but is applied to inform different questions (investigative, legal, operational, policy)
- Various testing and analysis that is applied can provide various types and fidelity of answers, some of this is technology or knowledge limiting with respect to the source materials (much is unknown about microbes)
- Science can provide varying levels of useful information depending on the sample, but usually must be caveated
- Unlike human DNA analysis, with which the source of a sample can be determined to a high degree of scientific certainty, or with fingerprint that it is accepted that it can (not proven), microbial forensics cannot achieve such at present (and may never be able to)
 - Toxin analysis is more limited to identification, with some exceptions/special conditions
- Deep microbial forensics for most threat agents has not been developed and validated as yet (useable on evidence “off the shelf”)
- Considerable gaps in the science and application exist, some of the science gaps are related to those that exist in other basic and applied science disciplines
- Like with other forensic disciplines, science alone will not “make the BW case” (probably never)

Framing Microbial Forensics “Grand Challenges”

- Significant scientific problems or needs which have been recognized and persisted for a considerable period of time with little or no progress
- Scientific or science-informed gap for which a solution would provide a considerable leap in “operationally useful” capabilities and knowledge
- Significant opportunity, which if developed, tested and implemented would provide huge boon to the investigation and prosecution of events involving biological threat agents, and possibly hold perpetrators at increased risk for attribution

Some “Microbial Forensics Grand Science Challenges”

- High Confidence Discernment of Natural, Accidental and Deliberate Similarly-Presenting Outbreaks, Approaching $t = 0$
- Establishing the Limits of What Current and Near-Term Microbial (Forensic) Characterization Methods are for the Identification of Priority Threat Agents Below the Strain/Isolate Level
- Very Rapid and Agile Development and Validation of New Highly Exploitative Forensic Analytic Methods in Response to “Surprise”
- Sampling and Forensic Characterization of Relevant Microbial Background On Demand on an Operational Time Scale
- Greater Forensic Exploitation of Biotoxins
- Agreed-to international microbial forensics quality standards
- International Microbial Forensics Data Sharing Forums and Standards
- International Repositories or Distributed Access to Reference Materials

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Designing an Effective Microbial Forensics Program for Law Enforcement and National Security Purposes

Randall S. Murch

Design Principles:

- Forensic Capabilities Should Help to Answer Key Investigative, Legal, Operational and Policy Questions
- Microbial Forensics Capabilities Should Coincide with Accepted Understanding, Uses and Expectations for Forensic Science
- Microbial Forensic Capabilities Should Incorporate Best Practices, Quality Measures, and Pertinent Science and Technology from Related Fields
- Forensic Capabilities Should Be Dynamic and Agile, and Adaptive to New Science When It Would Add Value

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Randall S. Murch

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Abstract Forensic capabilities that provide lead information, and investigative, intelligence, prosecution and policy decision support can be invaluable for responding to and resolving bioterrorism events. Attributing biological attacks through scientific and other resources and processes is an important goal for which science can be instrumental. Some even believe that having effective microbial forensics capabilities along with others can even deter adversaries from using biological weapons. For those nations that do not have such or wish to integrate or upgrade capabilities, thoughtful analysis and consideration of certain design principles will increase the likelihood that success will be attained.

Keywords Microbial forensics and attribution · Program design principles

Introduction

A new forensic investigative capability was launched in the United States in 1996 to support bioterrorism and vaccine investigations. That new program was created in the U.S. Federal Bureau of Investigation Laboratory in concert with the agency's emerging operational and investigative

IAP Scientific Seminar “Natural or Deliberate Outbreak—How to Detect or Deter and Trace its Origin: Biomedical, Surveillance, Forensic” organized by the Polish Academy of Sciences and the Military Institute of Hygiene and Epidemiology on 4 December 2013 in Warsaw.

R. S. Murch (✉)
Vigyan Politechnic Institute and State University, Arlington, VA, USA
e-mail: rmurch@vcc.edu

responsibilities with bioterrorism, and growing interest and concern in the U.S. at many levels with regards to this worrisome and complex threat. From the outset, this program drew on existing scientific, biomedical and civil support capabilities in the military, emergency response, national laboratory, public health and agricultural protection agencies. What resulted leveraged these into one system which truly had forensic investigation as a priority as well as sought to incorporate legal, operational and policy requirements. From this national effort, the scientific practice and communication of what became the field of *microbial forensics* was born. Since that time, a number of other nations have created their own versions of capabilities from what originated in the U.S. The science of microbial forensics and these disciplines upon which it was built and those upon which it is being advanced transcends national boundaries, governmental systems, priorities and applications.

Definitions

In order to provide common terms and perspectives for this paper, these definitions are offered for context:

- **Forensic Science:** The collection, preservation, transport, analysis, interpretation and reporting on physical evidence to determine its relevance to events, people, places, tools, methods, processes, intentions, plans involved in events pertaining to criminal, civil or international law. Of principal focus is the identification and characterization of evidence and determine its value and utility to answering key investigative and legal questions which lead to legal action (i.e. indictment, prosecution, conviction) or exoneration. dx.doi.org/10.1007/s00030-014-0209-7

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Perspective Article

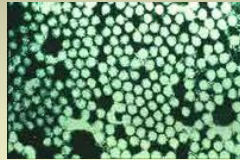
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Bioattribution needs a coherent international approach to improve global biosecurity

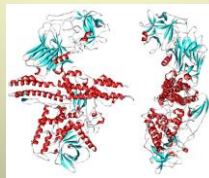
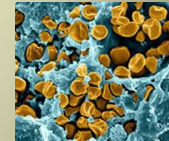
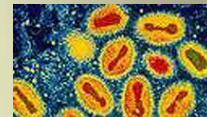
[Randall Steven Murch*](#)

*School of Public and International Affairs, Virginia Tech Research Center, Virginia Polytechnic Institute and State University, Arlington, VA, USA

- **Introduction**
- **Context and Background**
- **Preparing and Integrating Essential Elements for Effective Programs**
 - *Properly Staffed Teams, Coordinated Investigation, Information Access, Analysis, and Sharing*
 - *Technical Forensic Capabilities*
 - *Legal Process and Decision Making*
 - *Policy Process and Decision Making*
- **Moving Forward: Building the Base toward Broad Implementation**
 - *Recommendation 1: Increasing Awareness and Engagement*
 - *Recommendation 2: Country Assessments to Inform Investments*
 - *Recommendation 3: Integrated Training and Cross-community Exercises*
 - *Recommendation 4: Pilot (Demonstration) Efforts*



Questions?
Discussion?



Randall Murch, PhD
rmurch@vt.edu
1.571.858.3080 (office)

Back Up Slides

Designing the MF Program: Design Principle 1

- Forensic Capabilities Should Help to Answer Key Investigative, Legal, Operational and Policy Questions to Shape Decision Making
 - Science Should Provide Useful Information to Decision Makers at All Levels
 - Decision Makers Should Be Able Understand and Use What is Being Provided, Including Attributes and Limitations
 - Science is One of Several Key Components that are Necessary to Have in Place
 - Decision Making Occurs at the Tactical, Operational and Strategic Levels and Is Dynamic, So Capabilities That Support Must Be Too

MF Program: Design Principle 2

- Microbial Forensics Capabilities Should Coincide with Accepted Understanding, Uses and Expectations for Forensic Science as Well as Pertinent Science Disciplines
 - Classical Forensic Science has been in Existence for Many Decades, Microbial Forensics is a Young Discipline and Many Gaps in Knowledge and Capabilities Exist
 - Microbial Forensics Can Add Value to Ongoing Investigations and Decision Making But Has Limits to What it Can Provide
 - Some of the Analytic Methods and Technologies are Common to Other Fields & Uses But Are Used to Address Different Questions and Purposes in Microbial Forensics
 - Like Other Forensic Disciplines, Microbial Forensics has Requirements for Forensic Validation to Maximize Confidence for Performers and Customers

MF Program: Design Principle 3

- Microbial Forensic Capabilities Should Incorporate Best Practices, Quality Measures, Pertinent Science and Technology and Capabilities from Related Fields
 - Meets Requirements and Expectations of Sound Science
 - Meets Requirements and Expectations of Properly Developed, Validated and Applied Forensic Science; Can Survive Independent Review and Scrutiny
 - Is Tightly Connected to Investigative, Legal, Intelligence and Policy Questions and Needs
 - Can Be Communicated and Understood By the “Customer”, Including Limitations (as with Forensic Science)

MF Program: Design Principle 4

- Forensic Capabilities Should Be Dynamic and Agile, and Adaptive to New Science When It Would Add Value
 - Increase Speed and Reduce Time, “Discovery to Decision”, Aid in Reducing Risk and Increasing Success
 - Provide Kind and Quality of Answers When Needed During Investigative/Operational and Legal/Policy Cycles
 - Incorporate New Science and Technology When It Increases Confidence, Time-to-Answer, Exploitation of Available and Useful Information in Evidence

Bioattribution: Recommendation 4: Pilot (Demonstration) Efforts

- Deeper, hands on training for new technical capabilities could be provided to nations that have or can quickly implement basic capabilities.
- Collaborative research by multinational teams focused on “microbial forensics grand challenges”
- International agreement on establishing and accepting guidelines and standards for the practice of microbial forensics
- Developing and testing attribution decision frameworks would develop from structured conversations among legal and policy experts, augmented by technical experts