

# Exploring an International Microbial Forensics Capability to Support Attribution and Advance Global Biosecurity

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of the U.S. National Academy of Sciences**

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# Introducing Speaker & Topic

## ■ Speaker

- PhD, Life Sciences
- 23 Years, US Federal Bureau of Investigation (FBI, 10 years in FBI Laboratory), 1980-2002 (retired 2002)
  - Investigator, Field Supervisor
  - Practicing Forensic Scientist, Forensic Lab Senior Executive
  - Created the U.S. national WMD forensics program while in FBI (1996)
  - Major case and special events experience (terrorism)
- Professor at public university, Virginia USA since 2004
- Continues to publish and work in area, among others

## ■ Presentation

- **Introduce Forensic Science in Context**
- **Provide Foundation for Microbial Forensics (aka Bioforensics)**
- **Initiate Discussion to Explore and “Test” Microbial Forensics in Global Biosecurity Context**
- **Describe Relationship of Science to Law & Policy**
- **Catalyze Discussion on a Possible Path Forward**

# Global Leaders' Questions

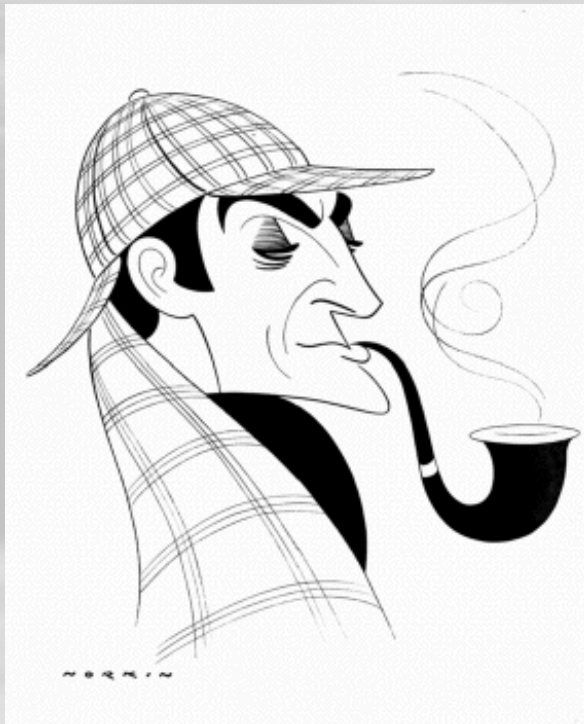
- What is or was it?
- How bad are the effects, and how much worse will it get?
- Who did it?
- Did it come from a lab we know about?
- Will there be more attacks?
- What are we and the nations involved doing about it?
- What can we know when, and with what confidence?



Consequence  
Mgmt

Crisis  
Mgmt

# *Science Can Help Answer Key Investigative, Legal & Policy Questions*



- Did A Crime or Event of Interest Occur?
- What Happened?
- How Did It Occur?
- When Did It Occur?
- Where Did It Occur?
- Why Did It Occur?
- **Who Was Involved?**
- **Who Was Responsible?**
- What Evidence Exists? What Does It Tell Us? How Strong are the Links?
- How Reliable and Credible is the Evidence?
- What are Alternative Explanations for the Evidence?
- Can We Defend Our Conclusions and Actions?

Science Can Help Inform Answers to These Questions,  
Which Can Lead to **Attribution**

# What is Forensic Science?

- Application of science in the investigation of legal (and policy) matters
- Scientific knowledge and technology are used to serve as independent “witnesses” commonly in criminal or civil matters, now in policy as well
- Science may not offer definitive solutions for all scenarios; it often does provide a special investigative role
- Goal is “attribution” – that is, who committed the offense

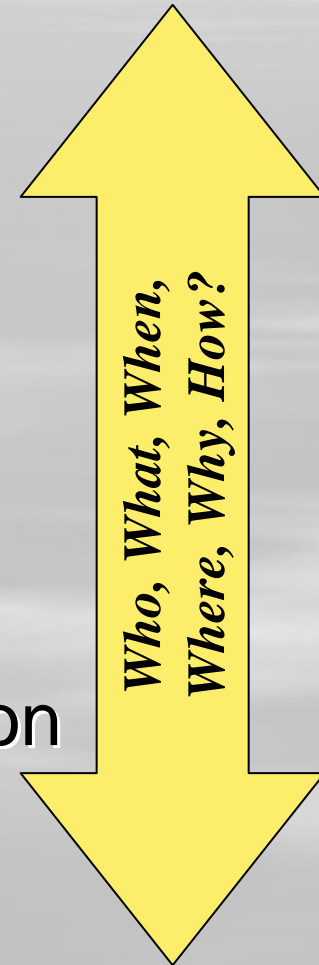


# Forensic Science is One Contributor to Attribution Decisions & Actions Following

- Attribution (Who did it?) is normally a “rule in, rule out” process during an investigation, in context of event and persons in question, and probative information & evidence
- If successful or allowed, science narrows potential sources of physical evidence while excluding others; context is important
  - Confidence in science adds to probative value and weight
- Investigative information, intelligence and science are leveraged dynamically to answer key questions, test theories and hypotheses, and arrive at likelihood of occurrence and value
- Sum of information and interpretation contributes to decision process and outcome

# Forensic Science Can Support & Help Direct Investigations

- Intelligence & Information Gathering
- Field Investigation, Evidence Collection
- Crime Scene Investigation
- Laboratory Analysis
- Interpretation, Integration, Application
- Building, Shaping Investigation/Prosecution (Defense)
- Communication and Decision Making



# Seeking Common Terms: Forensics & Attribution

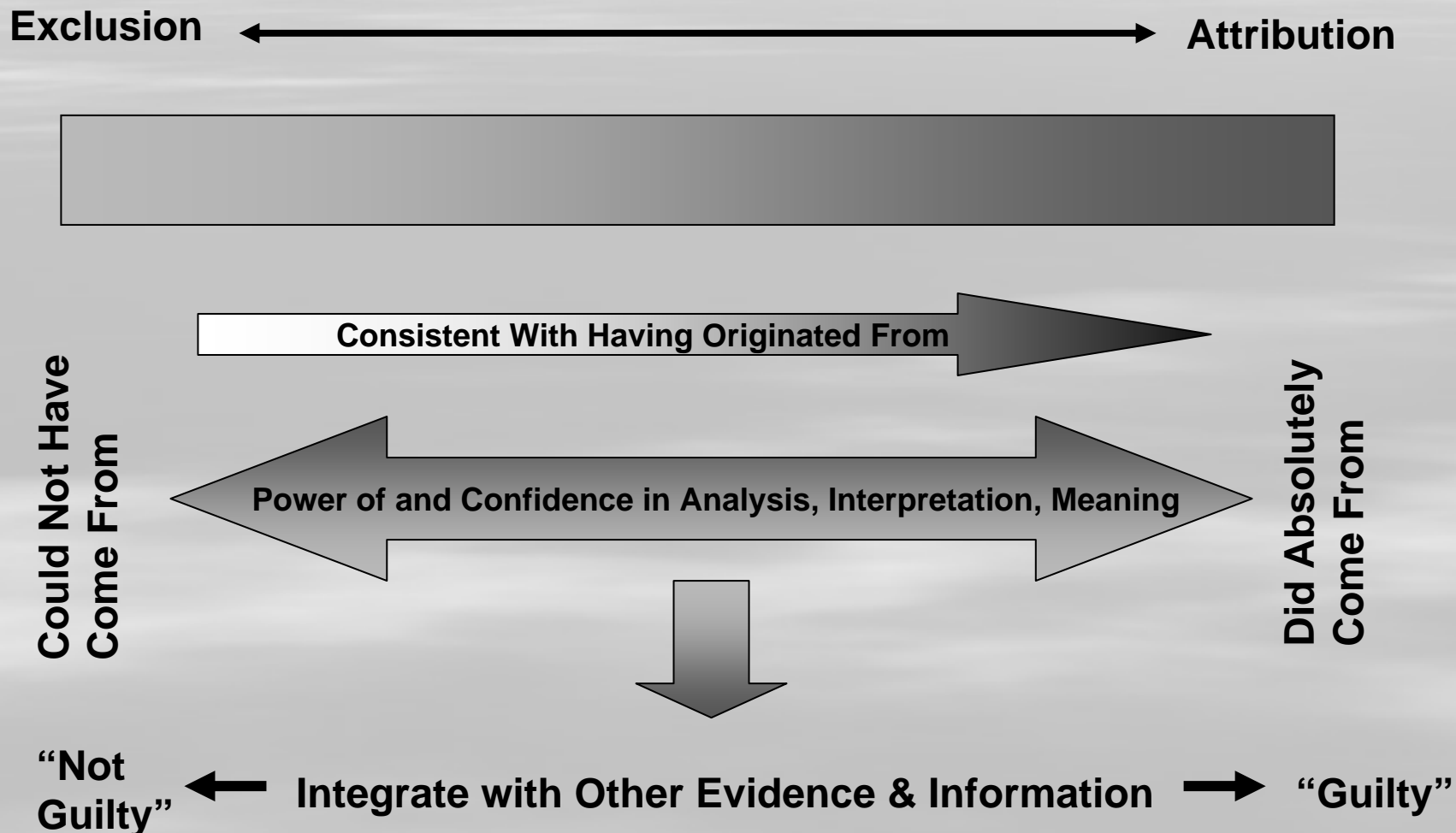
- **Forensics:** Analysis and interpretation of physical evidence to determine relevance to events, people, places, tools, methods, processes, intentions, plans
  - *Identification and Characterization*
  - *Inclusion toward Attribution, or Exclusion*
- **Attribution:** Assignment of a ***sample of questioned 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 “Q” with “K”
  - *Increasingly and more intensely, the science underlying “attribution” is being scrutinized, expectations are increasing for accuracy, reliability, validity*

The “definitions” and “standards” for attribution from the scientific, legal, policy and political perspectives differ!



# ***“The Forensic Continuum”***

*Individual & Compiled Sample  
Collection, Analysis & Interpretation*

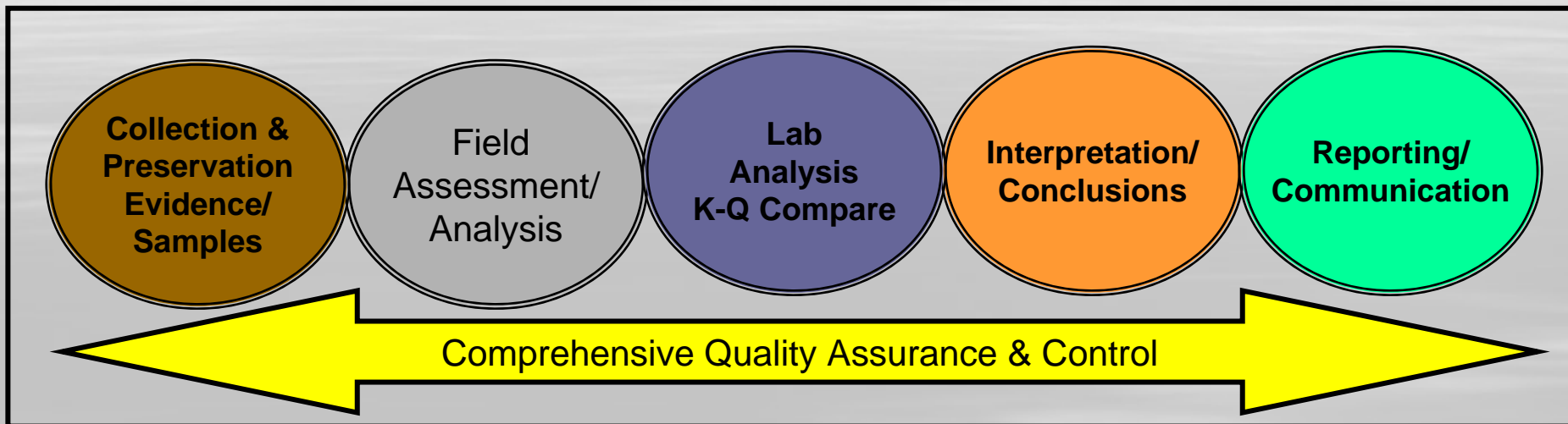


# Forensic Disciplines Include

- Biology/DNA
- Chemistry
- Materials
- Trace Evidence
- Impression and Pattern
- Engineering
- Digital-Computer
- Reconstructive
- Medicine



# 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

# Expectations for Contemporary Forensic Science are Increasing

- In the U.S. and elsewhere science for justice is being increasingly scrutinized for:
  - Accuracy
  - Reliability
  - Validity
  - Credibility
  - Repeatability
  - Defensibility

In the United States and other countries, forensic science and its performers have been shown or assessed to have significant gaps, shortcomings and needs:

- Scientific basis and validation of the science or practice in some disciplines
- Credentials and training of performers
- Funding and Infrastructure
- Organizational Independence
- Understanding, Use and Scrutiny by Legal and Judicial Communities

***See “Strengthening Forensic Science in the United States: A Path Forward” National Research Council (U.S.), 2009***

# Why “Quality” is So Important

- Increases confidence of those who rely on results, interpretations, findings and conclusions
- Sets high expectations for performers, services and outputs provided, interested or dependent organizations and stakeholders
- Ensures that best science and practice occurs
- Establishes culture of accountability, vigilance and continuous improvement within laboratories and with other practitioners



# Microbial Forensics is a Newer Forensic Discipline

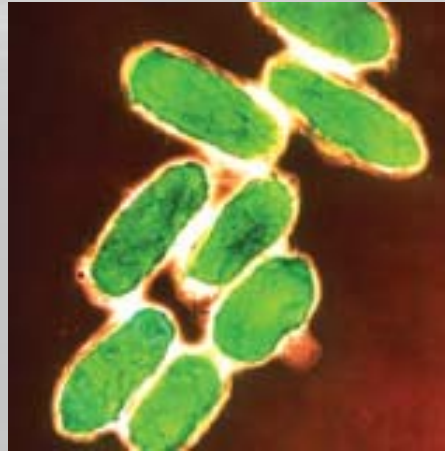
*Application of Forensic Science and Related Sciences to Problems Involving Biological Threat Agents, Their By Products and Associated Physical Evidence*



*What is It? Is it Probative/Relevant?  
Can It Be Linked to A Source?  
How Robustly & Precisely Can It Be Linked?  
What is the Meaning & Weight of the Conclusion?*

**Sample from  
A Questioned  
Source (Q)**

**Q**



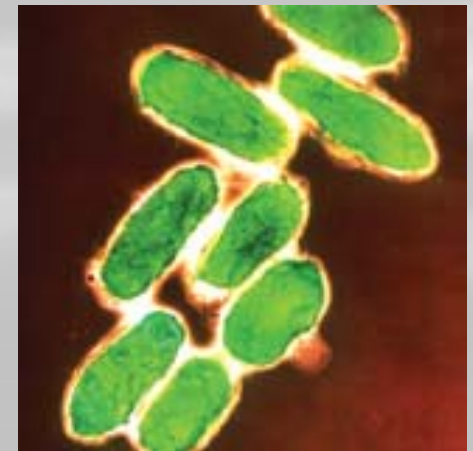
**Identity?**

**Relevance to Event?**

**Power of Methods to  
Characterize - Discriminate?**

**Confidence Limits?**

**Known Source (K)**

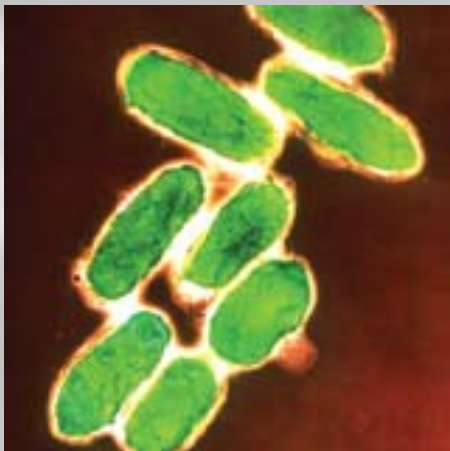


**Side by Side Comparison**

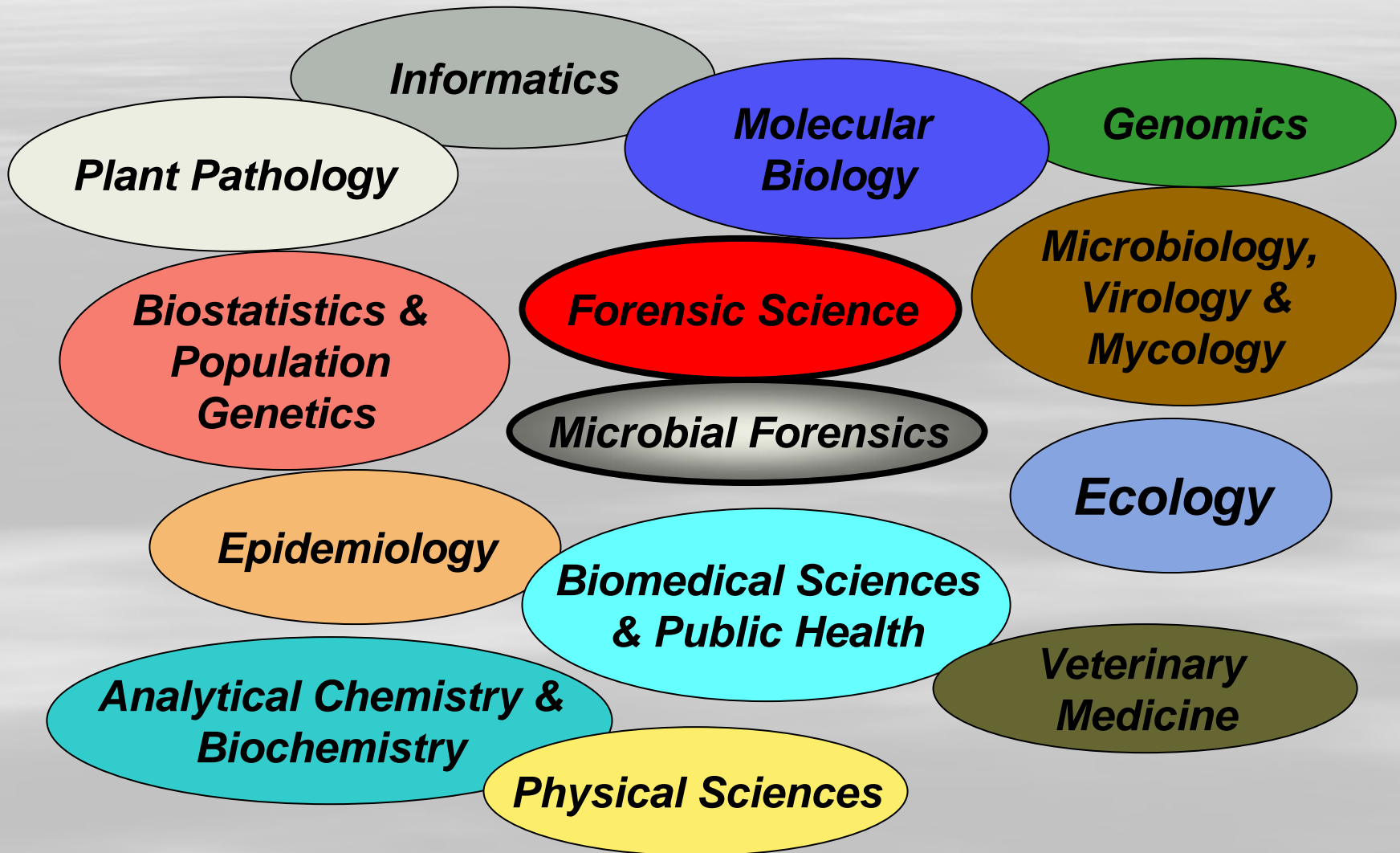
**Could Not Have Originated From**

**Consistent With Having  
Originated From (Weak →  
Strong)**

**Absolutely Did Originate From**



# Intersection of Disciplines



# Agents Affecting Human Health or Animal Health

|                            |                                |                                   |                                  |                                     |
|----------------------------|--------------------------------|-----------------------------------|----------------------------------|-------------------------------------|
| <b>Anthrax</b>             | <b>Psittacosis</b>             | <b>S A Hemorrhagic Fevers</b>     | <b>Avian Tuberculosis</b>        | <b>Duck Hepatitis virus</b>         |
| <b>Foot &amp; Mouth</b>    | <b>Sheep and Goat Pox</b>      | <b>Shiga-like RIP</b>             | <b>Bovine cystercercosis</b>     | <b>Epizootic Lymphangitis</b>       |
|                            | <b>Rickettsia rickettsii</b>   | <b>Lumpy Skin Disease</b>         | <b>Dourine</b>                   | <b>Enzootic Bovine Leukemia</b>     |
| <b>Brucellosis</b>         | <b>C perfringens toxin</b>     | <b>Shigellosis</b>                | <b>Enterovirus Encephalitis</b>  | <b>Equine Piroplasmosis</b>         |
| <b>Rift Valley Fever</b>   | <b>Rabies</b>                  | <b>Hepatitis E</b>                | <b>Equine Infect Anemia</b>      | <b>Fowl Pox</b>                     |
| <b>BSE</b>                 | <b>Ricin</b>                   | <b>Fowl Cholera</b>               | <b>Equine Rhinopneumonia</b>     | <b>Marek's Disease</b>              |
| <b>Swine Fever</b>         | <b>SEB</b>                     | <b>T2- mycotoxin</b>              | <b>Infectious Bursal Disease</b> | <b>Hemorrhagic Septicemia</b>       |
| <b>Nipah</b>               | <b>E coli</b>                  |                                   | <b>Maedi / Visna</b>             | <b>Horse Mange</b>                  |
| <b>Hendra</b>              | <b>VSV</b>                     | <b>Hydatid Disease</b>            | <b>Nairobi Sheep Disease</b>     | <b>Infect Bovine</b>                |
| <b>Botulinum</b>           | <b>Ebola/Marburg</b>           |                                   | <b>Paratuberculosis</b>          | <b>Rhinotracheitis</b>              |
| <b>Influenza</b>           | <b>Campylobacter</b>           | <b>Herpes B</b>                   | <b>Theileriosis</b>              |                                     |
| <b>Plague</b>              | <b>Bluetongue</b>              | <b>Abrin</b>                      | <b>Alastrim (Variola minor)</b>  | <b>Myxomatosis</b>                  |
| <b>VEE/WEE/EEE</b>         | <b>Lassa</b>                   | <b>Malignant Catarrhal</b>        | <b>Menangle</b>                  | <b>Ovine Pulmonary</b>              |
|                            | <b>Heartwater</b>              | <b>Fever</b>                      | <b>Avian Mycoplasmosis</b>       | <b>Adenovirus</b>                   |
| <b>Rinderpest</b>          | <b>Smallpox</b>                | <b>Peste des petits</b>           | <b>Bovine Anaplasmosis</b>       |                                     |
| <b>Glanders</b>            | <b>Swine Vesicular Disease</b> | <b>Avian Infectious</b>           | <b>Bovine Genital</b>            | <b>Porcine cystercercosis</b>       |
| <b>JE</b>                  | <b>Typhus</b>                  | <b>Bronchitis</b>                 | <b>Campylobacter</b>             | <b>Porcine resp &amp; rep syndr</b> |
| <b>NDV</b>                 | <b>Yellow Fever</b>            |                                   | <b>Contagious Agalactia</b>      | <b>Rabbit Hemorrhagic Dis</b>       |
| <b>Q Fever</b>             | <b>Akabane</b>                 | <b>Aujusky's Disease</b>          | <b>Dermatophilosis</b>           | <b>Surra</b>                        |
| <b>TBE</b>                 | <b>Shigatoxin</b>              | <b>Bovine Babesiosis</b>          |                                  | <b>Transmissible Gastroentero</b>   |
| <b>Tularemia</b>           | <b>Contagious Caprine</b>      | <b>Coccidiodes posadasii</b>      |                                  |                                     |
| <b>Hantavirus</b>          | <b>Pleuro.</b>                 | <b>Contagious Equine Metritis</b> |                                  | <b>Trichinellosis</b>               |
| <b>Salmonella</b>          | <b>Monkeypox</b>               |                                   |                                  | <b>Trichomonosis</b>                |
| <b>Microsporidia</b>       | <b>Dengue</b>                  | <b>Fowl Typhoid</b>               |                                  |                                     |
|                            |                                | <b>Pullorum</b>                   |                                  |                                     |
|                            |                                | <b>Conotoxin</b>                  |                                  |                                     |
| <b>CCHF</b>                |                                |                                   |                                  |                                     |
| <b>Bovine Tuberculosis</b> |                                |                                   |                                  |                                     |
| <b>African Horse</b>       |                                |                                   |                                  |                                     |
| <b>Sickness</b>            |                                |                                   |                                  |                                     |
| <b>Melioidosis</b>         |                                |                                   |                                  |                                     |

- Plant pathogens expand list substantially

- For vast majority, the forensic analysis has not been worked out or validated to date

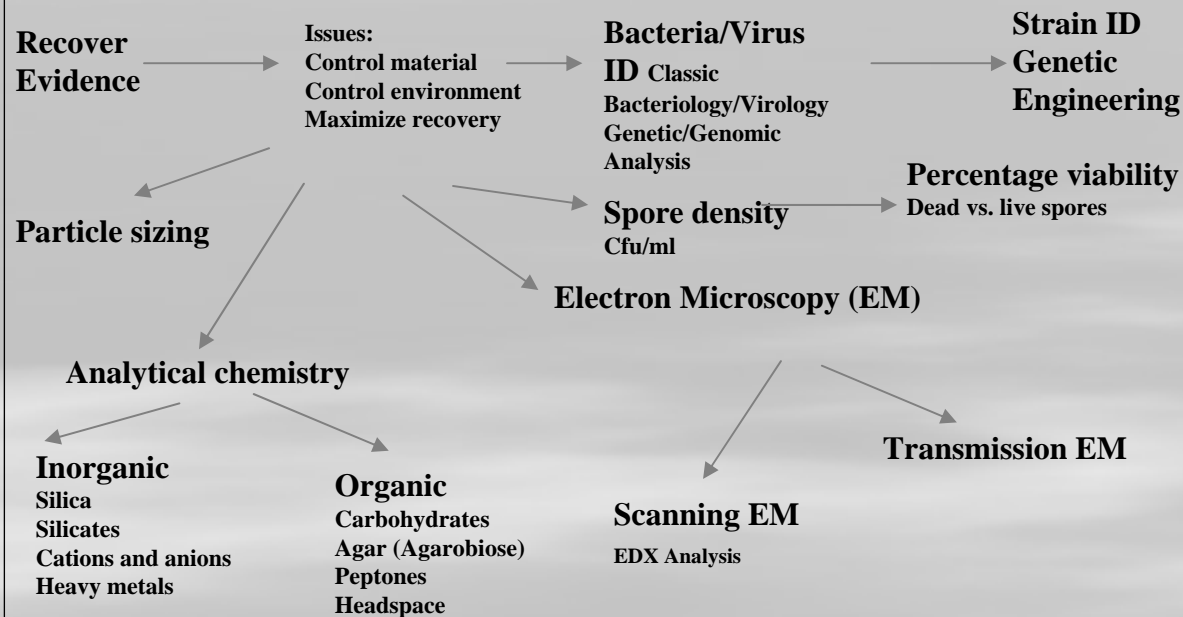
Derived from Various Sources



# ***General Analytic Approach***

## ***One View & Issues***

### **Conceptual Analytic Pathway for Microbial Evidence**



#### **But, Don't Forget!**

- Sample Type, Matrix, Situation & Question Dictate Methods/Protocols

- Traditional Physical Evidence

- Contaminated Traditional Evidence

- Good Intelligence and Investigation

- Apply Proper Interpretation

- Validated Methods by Qualified, Certified Personnel

- Environment of Robust Quality Assurance and Control

- Integrate Forensics into Investigation



# Clinical and Genomic Methods

- **Clinical Microscopy (Morphology: Size, Shape, Staining Characteristics)**
- **Clinical Culture Methods**
- **Metabolic Profiling:** Selective Biochemistry, Biolog™
- **Protein-Based Methods** Enzyme Linked Immunosorbent Assay, Serotyping, Antigen Capture, Matrix Assisted Laser Desorption Ion (MALDI) Time-of-Flight (TOF) Mass Spectroscopy, Multilocus Enzyme Electrophoresis (MLEE)
- **DNA Typing**
  - Restriction Enzyme Methodologies:* Restriction Fragment Length Polymorphism (RFLP), Pulse Field Gel Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP)
  - PCR-Based:* Intergenic Spacer Regions (ISR), ARDRA, Random Amplification of DNA (RAPD) Analysis, Repetitive Element (REP-PCR), Variable Number Tandem Repeats (VNTR), Multilocus Variable Number Tandem Repeat Analysis (MLVA), Inserted Sequence (IS) Elements, Triangulation Identification for Genetic Evaluation of Risk (TIGER, PCR-Mass Spectroscopy)
  - Hybridization:* Subtractive Hybridization, Microarray-Single Nucleotide Polymorphisms (SNP), Re-Sequencing, Gene Expression
  - DNA Sequencing:* Full Genome, Multilocus Sequence Typing (MLST)

# Physical and Chemical Methods

**Microscopy:** Scanning Electron Microscopy (SEM) without and with Energy Dispersive X-ray (EDX) Microanalysis, Atomic Force Microscopy (AFM)

**Spectroscopy:** Raman Spectroscopy, Surface-Enhanced Raman Spectroscopy

**Spectrometry:** Liquid Chromatography – Mass Spectrometry (LC/MS), Fourier Transform Infrared (FTIR) Spectrometry, Bio-Aerosol Mass Spectroscopy (BAMS), Matrix-Assisted Laser-Desorption Ionization Mass Spectrometry (MALDI-MS), Isotope Ratio Mass Spectrometry (IRMS), Time-of-Flight Secondary Ion Mass Spectroscopy (TOF-SIMS), Nano-Secondary Ion Mass Spectroscopy (nano-SIMS), Accelerator Mass Spectroscopy (AMS)

**Nuclear microscopy:** Particle-Induced X-ray Emission (PIXE) – Scanning Transmission ion microscopy (STIM)

# Existing and **Future** Contributions of Microbial Forensics

- Investigation & Prosecution of Suspected Criminal & Terrorist Events
- Joint Investigations with Public Health/Agriculture: Suspicious Outbreaks (Natural, Accidental, Deliberate)
- Resolving Hoax Cases
- Training & Exercises
- Investigating & “Prosecuting” Global BW Proliferation, Use?
- Supporting Global BW Non-Proliferation Initiatives?
- Contributing to More Robust Global Biosecurity Preparedness?
- Integrated Cooperation with Global Public Health, Veterinary, Plant Pathogen Biosurveillance?

All of these require an effective, credible, validated and defensible capability!

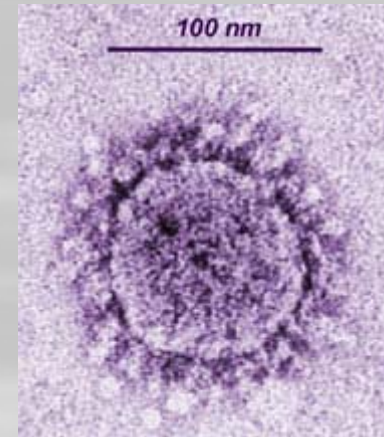
# Current State of Microbial Forensics and Use

- Prior existing, evolving scientific disciplines to draw from
- Certain U.S. Federal agencies have invested in science, infrastructure, personnel and equipment for field and analysis capabilities (since 1996, accelerated post-2001); now a accepted component of “biosecurity kit”
- Still a relatively young field
- Small number of other countries have shown interest, leveraging related national capabilities
- Substantial published science which supports forensic methods with some organisms and toxins; also published work that guides development, validation and setting expectations
- Knowledge and experience limited to certain priority organisms and toxins, still many gaps with those
- Forensics not yet developed and validated for many other potential BW agents
- Scientific attribution limited/difficult microbes and toxins
- “New science” presents unaddressed, unknown challenges
- Has never been formally scrutinized, challenged in court (U.S.)
- Science and applications undergoing assessment (U.S.)
- Legal frameworks for scientific evidence exist, policy frameworks do not
- Increased interest in the role of forensics in investigation, attribution, prosecution, exoneration in recent years, both from legal and policy perspectives (U.S., Others? BWC?)
- No global agreement on forensics applied to biosecurity response, policy and law, preparedness, cooperation

# ***Microbial Forensics:***

*Emerging and Future Technologies, Threats to Address?*

- ***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?*



# **Incorporating Microbial Forensics in Global Biosecurity Cooperation?**

- **Is there a formalized role for forensics within the BWC, other related programs?**
  - **How should it be defined? Who will define it? Who will set expectations and ensure these are met?**
  - **What is required to warrant building and properly using a high-quality forensic capability?**
  - **If it existed, how would the science interface with policy and law?**
  - **What is the process to achieve, maintain and sustain the desired capability and best value?**
  - **Who should oversee and manage? Who will resource and staff? Who will define its modus operandi?**
  - **How should it be “internationalized”? Confidence-building measures defined and incorporated?**
  - **How would it fit with other related programs and capabilities?**

# Key Elements to Establish & Validate?

- Initiate forensics into the “BWC and global biosecurity conversation” with experts
- Universal acceptance of forensics under the BWC
- Establish authorities, mechanisms
- Strategy, plan and priorities
- Designs and pathways to establish key elements and integration, measure progress, value and effectiveness
- Standards (guidelines) for scientific practice, development, acceptance, use, interpretation
- Frameworks for use of scientific outputs and testing in policy-legal decision making
- Identify initial and then establish sustaining expertise, infrastructure and resources
- Start with pilot/demonstration initiatives, workshops

# Policy and Legal Cooperation is Also Required

- Forensic science is not developed and used in a vacuum
- Successful investigations, actions or initiatives will not be based on science alone
- Science supports legal and policy processes and decisions
- Legal and policy requirements shape and define the science that is needed, and how it is used and tested



# Our Path Forward Together?

- **Develop a Strategy within the BWC framework and move forward with demonstration initiatives, which could include:**
  - **Identify “grand science challenges”**
  - **Establish standards/guidelines for collection, preservation, analysis and reporting and interpretation of results for priority organisms, toxins and sample types**
  - **Accept quality management standards (ISO)**
  - **Provide standardized introductory and advanced training, personnel certification protocols for voluntary or selected participants**
  - **Establish one or more accredited UN-authorized microbial forensics laboratories based on international quality management standards, and modus operandi to include transparency**
  - **Establish accepted sample sharing, analysis protocols**
  - **Establish an international microbial forensics repository which leverages existing, related resources, to include specimens which help to describe relevant geotemporal microbial background**
  - **Develop and validate legal & policy requirements for microbial forensics capabilities that support UN-sponsored investigations, and then criteria and guidelines for use of science in UN actions**

