Microbial Forensics and Law Enforcement

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Topics

- Introduction
- Relationship with Public Health (similar for Ag)
- Relationship with NBFAC
- QA & Validation
- Conclusions

Law Enforcement and Biothreat

- We all have responsibilities to prevent or at least limit any threats that may occur via the misuse of biotechnology
- Protect individuals and communities against such threats
- Deterring, interdict, respond to, and investigate criminal acts
- Prevention requires a framework of deterrents including awareness and detection of threats and investigation and attribution of criminal acts
- Essential to recognize that no single approach will be 100% effective
- Strongest and most effective framework will require integration of responsibilities and capabilities across individuals, commercial organizations, private and non-governmental organizations, and government

Law Enforcement and Biothreat

- Responsibilities include providing forensic evidence for convictions and investigations
- The FBI is lead agency responsible for investigating violations that may be terrorist in nature as defined in Weapons of Mass Destruction (WMD)-related statutes
- Any threatened use of a disease-causing organism directed at humans, animals, or plants is a crime, regardless of whether the perpetrator actually possesses a disease-causing agent

Law Enforcement and Biothreat

- Biological Weapons Anti-Terrorism (BWAT) Act revisions contained within the USA PATRIOT Act establish that knowingly possessing a biological agent, toxin, or delivery system that cannot be justified by a prophylactic, protective, bona fide research, or other peaceful purpose can result in arrest, prosecution, and fines or imprisonment of up to ten years

- Individuals and organizations in possession of potentially harmful biological agents document that they have such material for legitimate purposes

Law Enforcement and Biothreat

- Select Agent Rule (SAR) is central to prevention and investigation of misuse of potentially harmful biological agents
- The SAR enables the monitoring and tracking of specified pathogens and toxins (i.e., “select agents”)
- The SAR applies primarily to the physical biological agent (e.g., an intact virus particle) and only to genetic material for a subset of select agents
- This subset is determined on the basis of whether or not the genetic material can be readily used to produce the intact and infectious agent itself or functional form of the toxin
- The SAR enables the arrest and prosecution of individuals who illegally possess or distribute such agents

Law Enforcement and Biothreat

- Response to an alleged incident begins with rapid and comprehensive assessment of the threat
- The FBI conducts a formal Threat Credibility Assessment
- The process draws upon a number of interagency experts, including HHS, CDC, and USDA
- The Threat Assessment process includes an analysis of technical feasibility, operation practicability, and behavioral resolve
- The results of this assessment are incorporated in the decisions involving the deployment of FBI response assets, the request, coordination and deployment of other U.S. government assets, and the notification of state and local authorities

Forensic Science

- Application of science in the investigation of legal matters
- Scientific knowledge and technology are used to serve as witnesses in both criminal and civil matters
- 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
- Microbial Forensics is multidisciplinary

Microbial Forensics

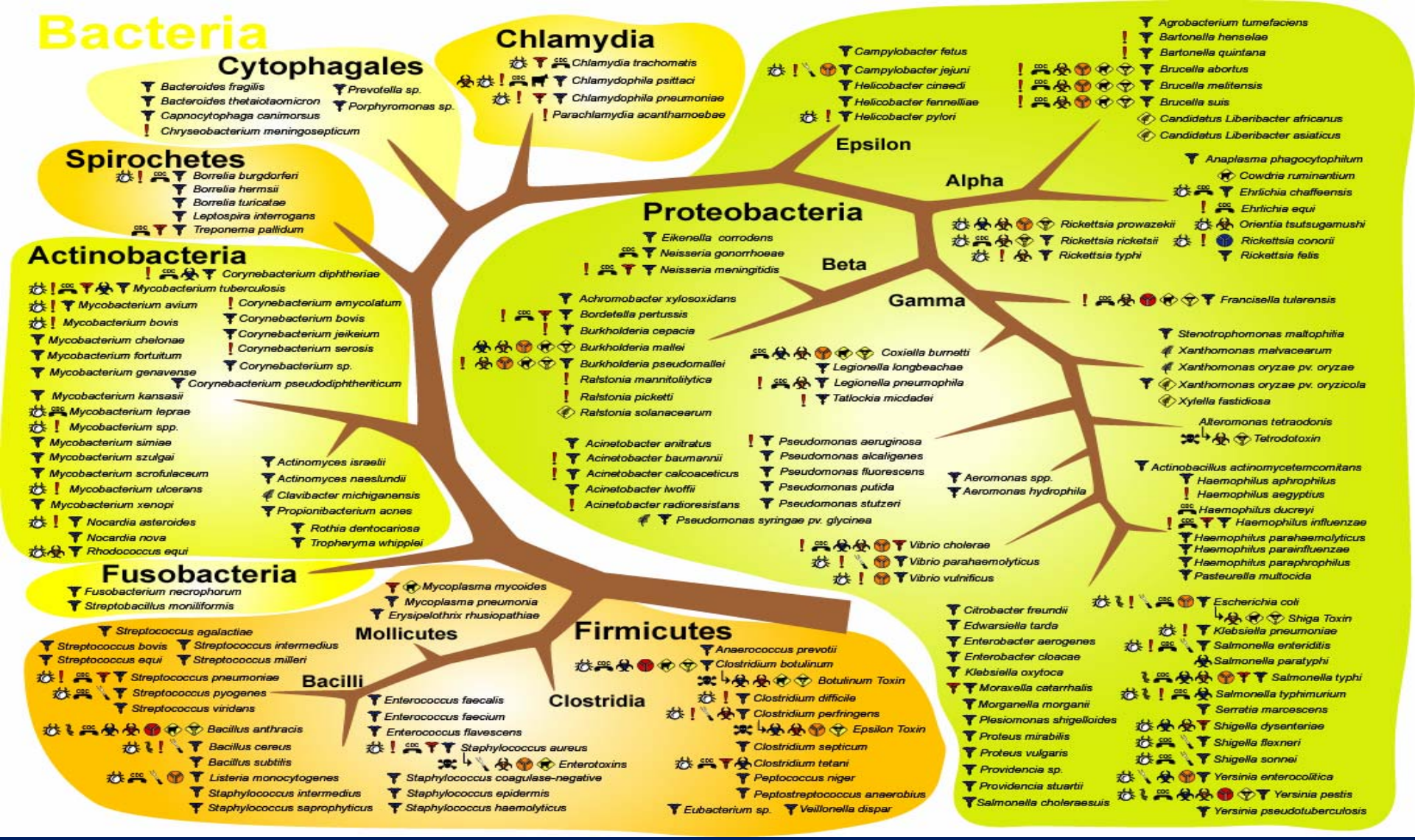
- Analysis of evidence from a bioterrorism act, biocrime, hoax, or inadvertent microorganism/toxin release for attribution purposes
- Essentially the same as any other forensic discipline

Current Situation

- The FBI is the lead investigative agency in response to acts of terrorism
- The FBI Laboratory currently can not conduct forensic examinations of hazardous CBRN agents at Quantico
- Robust capabilities for the forensic exploitation of hazardous CBRN evidence are lacking at other laboratories
- The knowledge base required to interpret and assign weight to some CBRN analyses is lacking

A Complex Problem

- Potential terrorist weapons include a wide range of chemical, biological, and radiological / nuclear agents
  - *Biological*:
    - Human, plant and animal Pathogens
  - *Chemical*:
    - Chemical warfare agents, toxic industrial chemicals
  - *Radiological / Nuclear*:
    - Actual material, dispersive device (RDD), low – high yield device
  - Must Consider ALL Related Evidence and Matrices


Bacteria
Cytophagales
Bacteroides fragilis
Bacteroides thetaiotaomicron
Capnocytophaga canimorsus
Chryseobacterium meningosepticum
Prevotella sp.
Porphyromonas sp.
Chlamydia
Chlamydia trachomatis
Chlamydophila psittaci
Chlamydophila pneumoniae
Parachlamydia acanthamoebae
Spirochetes
Borrelia burgdorferi
Borrelia hermsii
Borrelia turicatae
Leptospira interrogans
Treponema pallidum
Actinobacteria
Corynebacterium diphtheriae
Mycobacterium tuberculosis
Mycobacterium avium
Mycobacterium bovis
Mycobacterium chelonae
Mycobacterium fortuitum
Mycobacterium genavense
Mycobacterium kansasii
Mycobacterium leprae
Mycobacterium spp.
Mycobacterium simiae
Mycobacterium szulgai
Mycobacterium scrofulaceum
Mycobacterium ulcerans
Mycobacterium xenopi
Nocardia asteroides
Nocardia nova
Rhodococcus equi
Corynebacterium amycolatum
Corynebacterium bovis
Corynebacterium jeikeium
Corynebacterium serosis
Corynebacterium sp.
Corynebacterium pseudodiphtheriticum
Actinomyces israelii
Actinomyces naeslundii
Clavibacter michiganensis
Propionibacterium acnes
Rothia dentocariosa
Tropheryma whipplei
Fusobacteria
Fusobacterium necrophorum
Streptobacillus moniliformis
Proteobacteria
Epsilon
Campylobacter fetus
Campylobacter jejuni
Helicobacter cinaedi
Helicobacter fennelliae
Helicobacter pylori
Alpha
Agrobacterium tumefaciens
Bartonella henselae
Bartonella quintana
Brucella abortus
Brucella melitensis
Brucella suis
Candidatus Liberibacter africanus
Candidatus Liberibacter asiaticus
Anaplasma phagocytophilum
Cowdria ruminantium
Ehrlichia chaffeensis
Ehrlichia equi
Orientia tsutsugamushi
Rickettsia conorii
Rickettsia felis
Rickettsia prowazekii
Rickettsia rickettsii
Rickettsia typhi
Beta
Eikenella corrodens
Neisseria gonorrhoeae
Neisseria meningitidis
Achromobacter xylosoxidans
Bordetella pertussis
Burkholderia cepacia
Burkholderia mallei
Burkholderia pseudomallei
Ralstonia mannitolilytica
Ralstonia picketti
Ralstonia solanacearum
Gamma
Francisella tularensis
Coxiella burnetti
Legionella longbeachae
Legionella pneumophila
Tatlockia micdadei
Stenotrophomonas maltophilia
Xanthomonas malvacearum
Xanthomonas oryzae pv. oryzae
Xanthomonas oryzae pv. oryzicola
Xylella fastidiosa
Acinetobacter anitratus
Acinetobacter baumannii
Acinetobacter calcoaceticus
Acinetobacter lwoffii
Acinetobacter radioresistans
Pseudomonas aeruginosa
Pseudomonas alcaligenes
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas stutzeri
Pseudomonas syringae pv. glycinea
Aeromonas spp.
Aeromonas hydrophila
Alteromonas tetraodonis
Tetrodotoxin
Actinobacillus actinomycetemcomitans
Haemophilus aphrophilus
Haemophilus aegyptius
Haemophilus ducreyi
Haemophilus influenzae
Haemophilus parahaemolyticus
Haemophilus parainfluenzae
Haemophilus paraphrophilus
Pasteurella multocida
Vibrio cholerae
Vibrio parahaemolyticus
Vibrio vulnificus
Escherichia coli
Shiga Toxin
Citrobacter freundii
Edwarsiella tarda
Enterobacter aerogenes
Enterobacter cloacae
Klebsiella oxytoca
Moraxella catarrhalis
Morganella morganii
Plesiomonas shigelloides
Proteus mirabilis
Proteus vulgaris
Providencia sp.
Providencia stuartii
Salmonella choleraesuis
Klebsiella pneumoniae
Salmonella enteriditis
Salmonella paratyphi
Salmonella typhi
Salmonella typhimurium
Serratia marcescens
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Yersinia enterocolitica
Yersinia pestis
Yersinia pseudotuberculosis
Mollicutes
Mycoplasma mycoides
Mycoplasma pneumonia
Erysipelothrix rhusiopathiae
Firmicutes
Bacilli
Streptococcus agalactiae
Streptococcus bovis
Streptococcus intermedius
Streptococcus equi
Streptococcus milleri
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus viridans
Bacillus anthracis
Bacillus cereus
Bacillus subtilis
Listeria monocytogenes
Staphylococcus intermedius
Staphylococcus saprophyticus
Enterococcus faecalis
Enterococcus faecium
Enterococcus flavescens
Staphylococcus aureus
Enterotoxins
Staphylococcus coagulase-negative
Staphylococcus epidermis
Staphylococcus haemolyticus
Clostridia
Anaerococcus prevotii
Clostridium botulinum
Botulinum Toxin
Clostridium difficile
Clostridium perfringens
Epsilon Toxin
Clostridium septicum
Clostridium tetani
Peptococcus niger
Peptostreptococcus anaerobius
Eubacterium sp.
Veillonella dispar
NIAID Category A Priority Pathogen
NIAID Category B Priority Pathogen
NIAID Category C Priority Pathogen
HHS Select Agent
Globally Important Human Pathogen
Medically Important Human Pathogen
Validated Biological Weapon
Potential Biological Weapon
Validated Biocrime Agent
High Potential For Bioengineering
CDC Notifiable Agent
Emerging Infectious Agent
USDA High Consequence Animal Pathogen
Important Animal Pathogen
USDA High Consequence Plant Pathogen
Important Plant Pathogen
Zoonotic Agent
Toxin
Principal Foodborne Pathogen

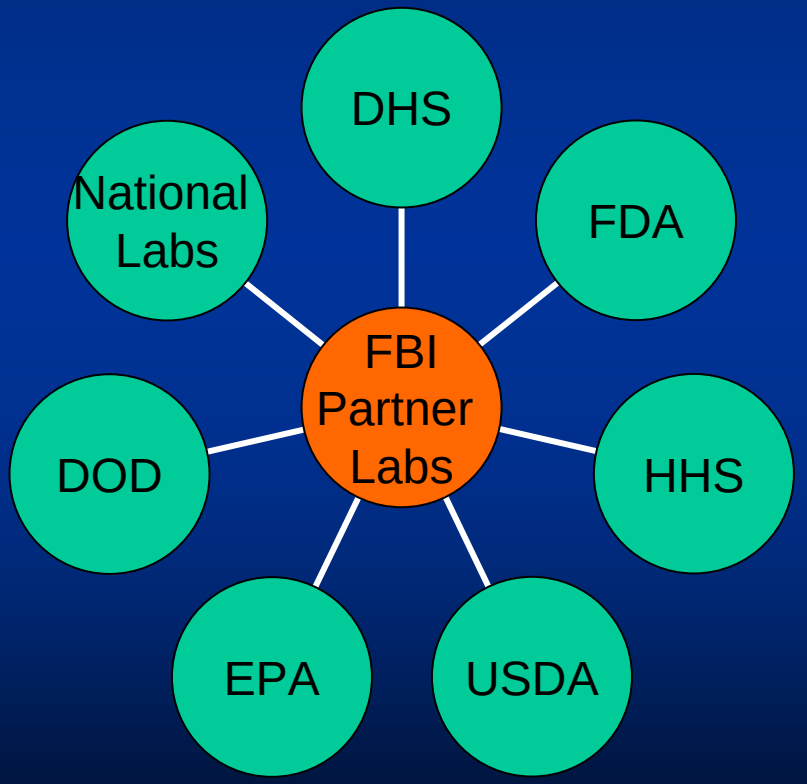
Proactive

Build relationship / infrastructure

The Solution

Success Through Partnerships

- Establish dedicated forensic CBRN facilities
- “Hub and Spoke Model”



The Solution:
Success Through Partnerships

- National BioForensics Analysis Center at Fort Detrick, Maryland is the FBI Partner Lab Hub for Biological Agents

Overt Attack

Courthouse receives letter labeled "Anthrax"

Covert Attack

Unusual, Disease Clusters, Signs & Symptoms

State & Local Public Health

↓ ↘

Law Enforcement ⟷ CDC/FDA


THEN
ATCHOO!
BLESS YOU
NOW
ATCHOO!
HELLO, FBI?

Stress --- Epidemiology and Forensics

- Build relationships with public health, agriculture and laboratory assets (government, academia, and private)

National system – sentinels, first detectors

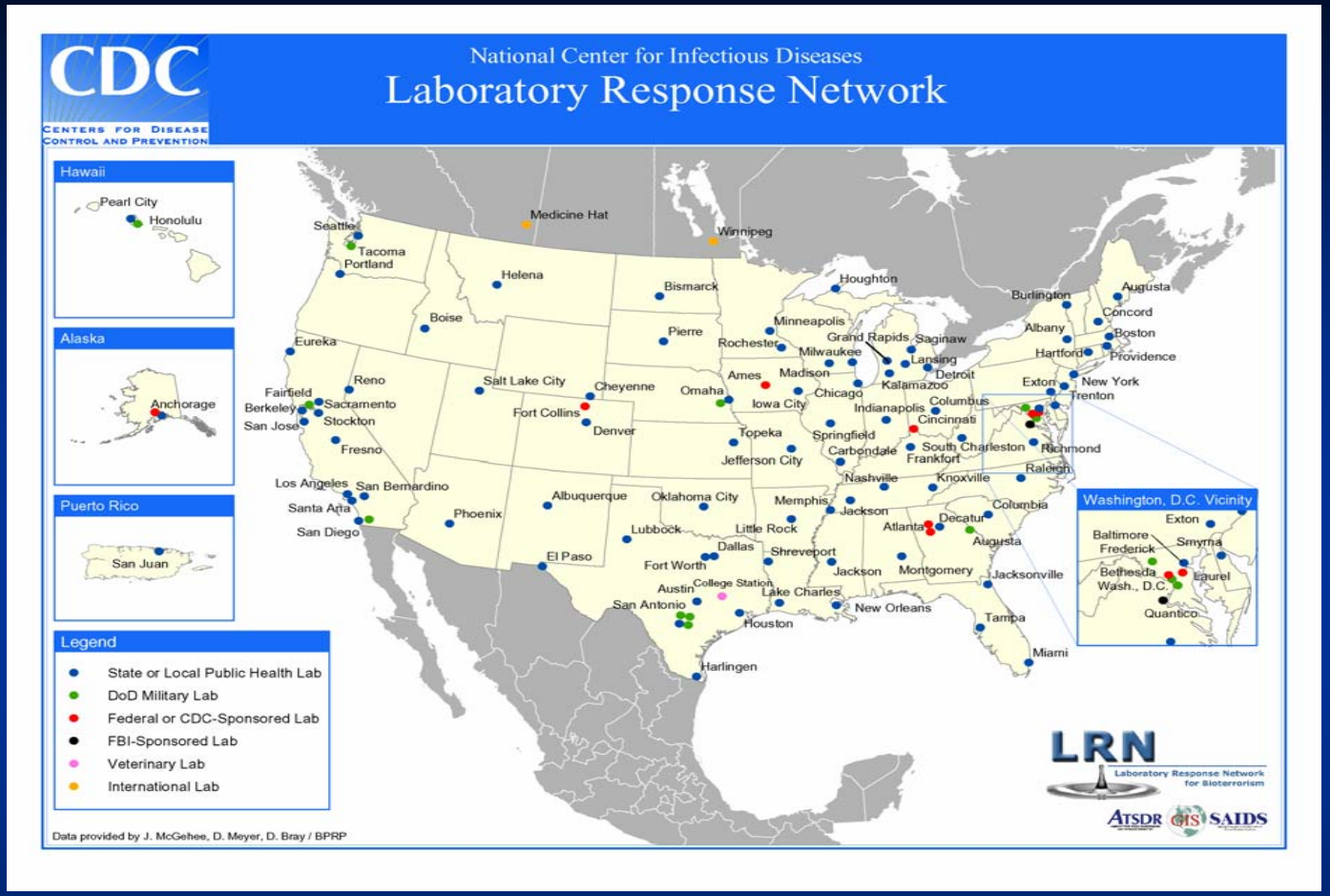
Epidemiologic considerations that may signal a bioterrorist attack

- Disease caused by an uncommon agent (such as smallpox)
- Unusual, atypical, genetically engineered or antiquated strain of agent
- High morbidity or mortality associated with a common disease or syndrome
- Failure of patients to respond to usual therapy
- Disease with an unusual seasonal or geographic distribution
- Increase in normal incidence
- Atypical disease transmission (such as shigella in muffins)
- Illness in people who are exposed to same ventilation system

Epidemiologic considerations that may signal a bioterrorist attack

- More than one unusual or unexplained disease existing in a person
- Illness that affects a large disparate population
- Illness that is unusual for a population or age group
- Unusual death or pattern of illness in animals preceding or accompanies death or illness in humans and vice versa
- A number of ill persons seeking treatment or medicine at the same time
- Same strain or genetic type from spatially or temporally disparate sources
- Simultaneous cluster of disease in noncontiguous areas
- Large number of unexplained diseases or deaths

Laboratory Response Network



Partnership between CDC & FBI serves at least three functions:

(1) Sampling mechanism for environmental samples
(2) Sentinel warning system for covert bio-events
(3) Technical resource

Relationship with Public Health

Covert Scenario

- LRN to CDC
- MOU - If unusual, notify the FBI
- Coordination of analysis
- CDC will analyze if have capability
- Recognizes ownership of evidence by law enforcement
- Limited sample – first priority public health
- Even public health analysis can have forensic value

Quality Management Guidelines for Laboratories Performing Microbial Forensic Work

- Goal is to promote development of a microbial forensics program that is scientifically valid and rigorous
- Define criteria for development and validation of microbial forensics methods that will support attribution for criminal investigations
- Establish national working guidelines for quality assurance and quality control as applied to microbial forensics

Validation

- Necessary
- Varies
- Ill defined
- Challenge to define better…
- Documents – QA & Validation

Validation

- Collection
- Shipping and storage
- Extraction
- Analysis
- Interpretation

Example Validation Criteria List

- Sensitivity
- Specificity
- Reproducibility
- Precision
- Accuracy
- Resolution
- Reliability
- Robustness
- Specified samples
- Purity
- Input values
- Quantitation
- Dynamic range
- Limit of detection
- Controls
- Window of performance for operational steps of assay
- Critical equipment calibration
- Critical reagents
- Databases

Note: Not all these need apply and others may be necessary

Validation of Testing Procedures

- Developmental Validation
- Internal Validation
- Preliminary Validation***

Validation

- Developmental validation is the acquisition of test data and determination of conditions and limitations. Developmental validation should be appropriately documented and should address specificity, sensitivity, reproducibility, bias, precision, false-positives, false-negatives, and determine appropriate controls. Any reference database used should be documented.

- Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods perform as expected

Validation

- Preliminary validation is the acquisition of limited test data to enable an evaluation of a method used to provide investigative support to investigate a biocrime or bioterrorism event. If the results are to be used for other than investigative support, then a panel of peer experts, external to the laboratory, should be convened to assess the utility of the method and to define the limits of interpretation and conclusions drawn.

- SOPs are for routine work
- But so locked in – restricts analytical thinking and possibly ignores both inculpatory and exculpatory evidence

Validation

- “Validation Plan” should be prepared
- To facilitate, guide, and educate
- Establish the range of conditions for which the interpretation of the analytical results is valid
- Equally as important, the conditions where results or the standard interpretation are not valid

Validation

A validation protocol should at a minimum include:

- Description of goal or purpose of the assay
- Critical steps
- Critical reagents
- Critical equipment
- Parameters and conditions to be evaluated
- Reference and test materials needed
- Sufficient number of replicate analyses to demonstrate reproducibility and reliability
- Aspects unique to the system that require specified validity testing

Minimum Validation Criteria

- Sensitivity
- Specificity
- Reproducibility
- Precision
- Accuracy
- Robustness
- Analysis of specified samples (e.g., reference panels and mock or non-probative materials) commensurate with the intended application of the assay

Collection Validation

- Recovery
- Stability
- Integrity
- Target – either organism or analyte (e.g., toxin)
- Influence of sample matrix

Collection Validation

- Sampling strategy – hypothesis or circumstance driven, targeted or randomized/statistical collection
- Describe controls such as field blanks
- Consider resource limitations
- Type of collection material or tool
- Efficiency of collection
- Efficiency of recovery
- Substrate - inert or interacts with the target
- Stability and preservation - inhibitors, viability…
- Recovery from matrix
- Packaging and storage strategies

Sampling Objectives

- Real-time monitoring
- Screening
- Bulk material
- Questionable article
- Extent of contamination
- Effectiveness of decontamination
- Clearance for re-occupancy
- Transitional
- Crime scene / forensic

Sampling Approach

- Logical and systematic
- Scheduled
- Risk-based
- Targeted
- Statistical/Random

Sample Collection and Preservation for Plant Pathogens

- Develop plan with consultation
- Use experience of current collectors for how to collect (pattern and cutting and bagging)
- Chain of custody
- Targeted and statistical sampling plans – symptomatic/asymptomatic
- Collect vectors
- Collection from multiple plant parts, multiple plants
- Collection from other nearby plants
- Stability over time
- Preservation and transport of sample material (“breathing” of bags)
- On ice - 4C? Glycerol? Dessicant?

Extraction of Analyte

- Specific – virus, bacteria, fugal, toxin
- Spore v vegetative cell
- Active v inactive (culture plan, amplification plan)
- Analyte that will be assayed– DNA, RNA, protein, Lipid, stabilizers, media, fatty acid, etc
- Stability of analyte
- Matrix effect – substrate, other co-extracted analytes, materials such as soil

Extraction of Analyte

- Maintenance of original state – activity (toxin)
- Minimum input
- Yield, recovery
- Purity
- Critical reagents
- Controls

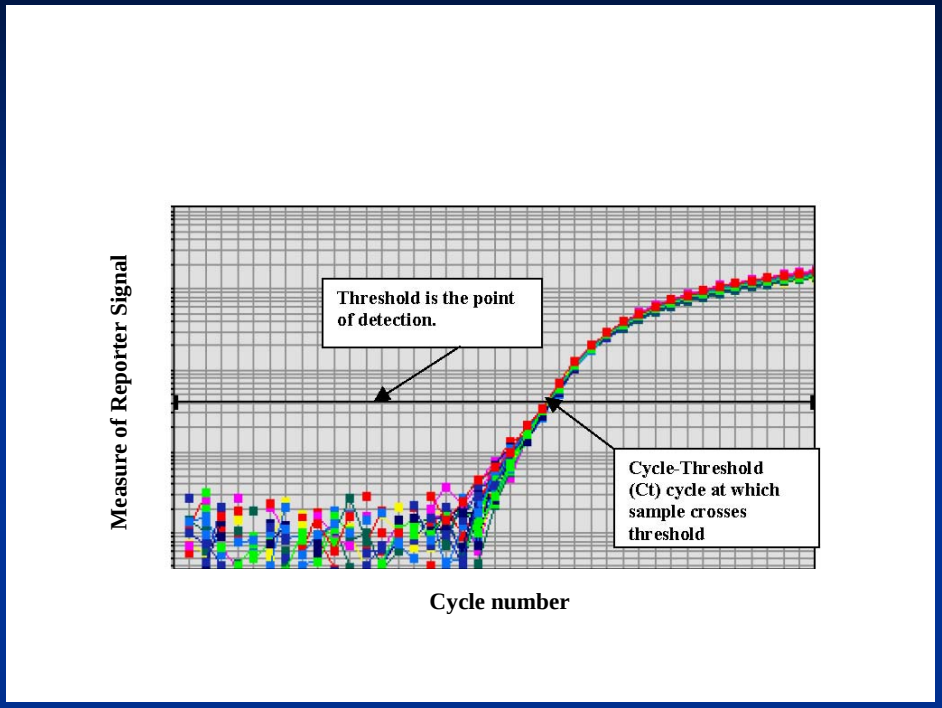
Analysis Phase Validation

- SOP
- controls – positive, negative and inhibition
- Specificity
- Dynamic range
- Reproducibility
- Reliability
- Precision
- Accuracy
- Predictive value

Analysis Phase Validation

- Input value
- Critical reagents
- Sensitivity
- Limit of detection
- Window of performance for operational steps of assay
- Critical equipment and calibration
- Interpretation criteria for results
- Resolve conflicting results

Interpretation



- Sensitivity claims of 1copy
- Is this reliable?
- What does it mean?
- Should there be an “inconclusive” category?

Interpretation of Results

- Statements – qualitative, quantitative, semi-quantitative
- Database – type, relevance, representative, quality of data
- Background data – normal values, reference range, endemicity
- Does a result require follow up or further analysis – temporal/spatial analysis, effect of passage

Interpretation of Results

- Alternate (reasonable) explanations
- Limits of interpretation
- Statistical approach – match, similarity, most recent common ancestor, identical
- Thresholds
- Software

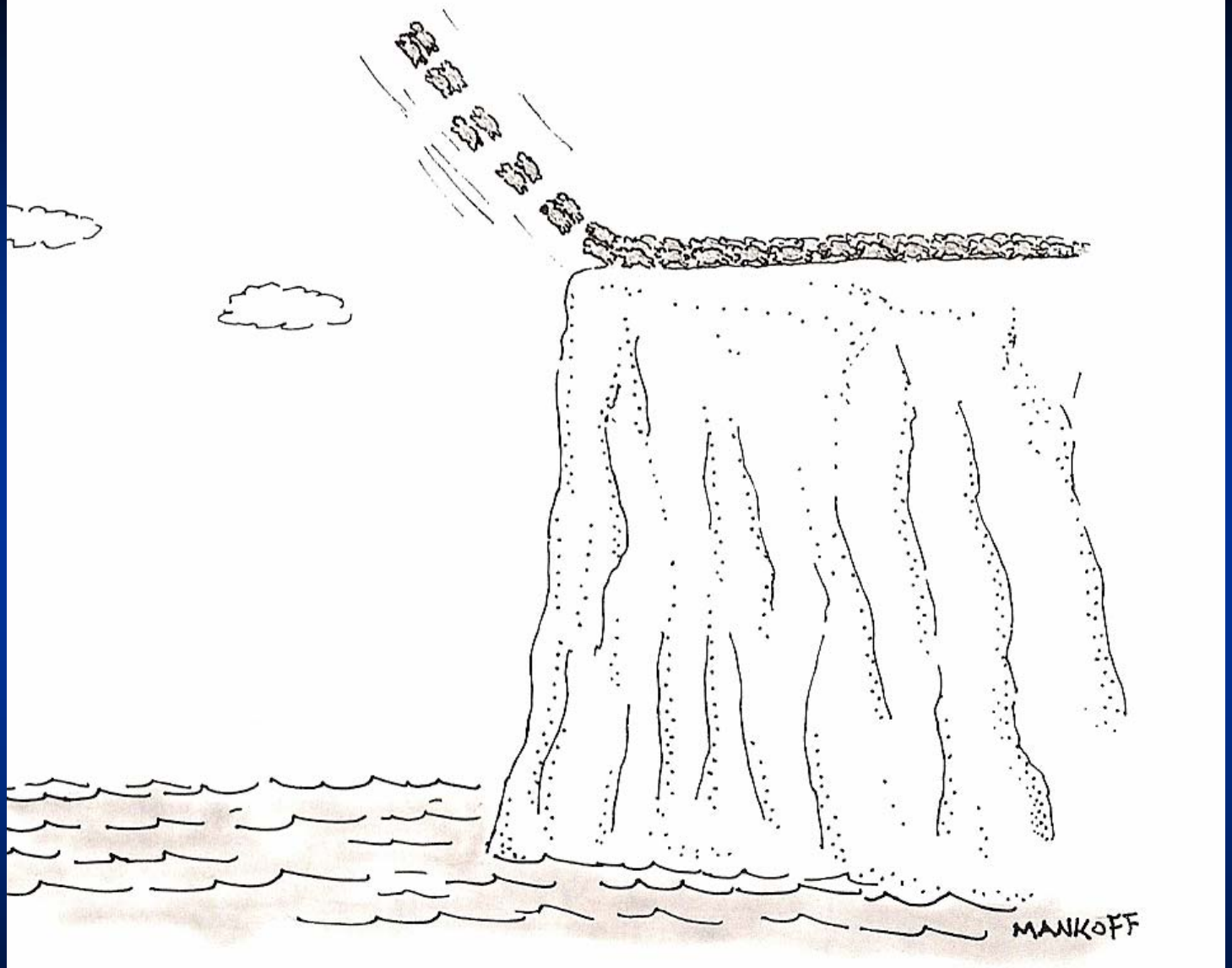
Standard Operating Protocols

- Contain sufficient detail about the procedure so one can carry out the assay and include, if appropriate:

  1) all steps in the procedure
  2) proper controls
  3) all reagents and preparations
  4) calibration
  5) criteria for analysis of results
  6) interpretation of results

Miscellaneous Needs

- Need a panel of isolates to test (validate) the assays (a “type” set)
- Type of panel materials? – appropriate to assay
- Validity of panel
- Literature compendium - especially of old literature



WHAT LEMMINGS BELIEVE

Forensic Questions

- What is the agent?
  - Species, strain, or more
- Was the event intentional?
  - Obvious in the Anthrax case
- How was it made?
- Where did it come from?
- Who did it?

Non DNA-based tools for the microbial forensics toolbox

- Characterization of physical attributes acquired during preparation
- Isotope analyses to approximate the age and source
- Physiologic methods (e.g., fatty acid composition, phage typing, serotyping)
- Analysis of growth media and media components adhering to the microorganisms
- Analysis of stabilizers and additives used in the preparation of a sample

Non DNA-based tools for the microbial forensics toolbox

- Identification of incidental biocontaminants, such as environmental pollen and fungi, for location and time of year of preparation

- Better understanding of bacterial endemism for identifying unique strains that may exist in only one location or few locations

- Monitoring changes in the immunological response of a host to a pathogen or toxin, such as temporal IgG and IgM responses

- Improvements in immunoassays (and antibodies) for more effective rapid detection and field deployable assays.

Forensic Genetic Questions

- What might be deduced concerning the nature and source of the evidentiary sample?
- Is the pathogen detected of endemic origin or introduced?
- Do the genetic markers provide a significant amount of probative information?
- Does the choice of markers allow the effective comparison of samples from known and questioned sources?
- If such a comparison can be made, how definitively and confidently can a conclusion be reached?

Forensic Genetic Questions

- Are the genetic differences to few to conclude that the samples are not from different sources (or lineages)?
- Are these differences sufficiently robust to consider that the samples are from different sources?
- Is it possible that the two samples have a recent common ancestor or how long ago was there a common ancestor?
- Can any samples be excluded as contaminants or recent sources of the isolate?
- Are there alternative explanations for the results that were obtained?

Comparison of Human DNA Forensics and Microbial Forensics

Similarities

- QA/QC
- Databases
- Qualitative conclusions of test results
- Quantitative conclusions
- General acceptance, admissibility,…

Differences

- Database size and composition
- Statistical Interpretation methods
- Confidence/uncertainty of outcome of interpretation

Components of a bioinformatic genetic toolbox for microbial forensics

- Algorithm(s) for DNA marker alignment encompassing pattern heterogeneity of the various types of genetic markers and for detecting genes, such as for pathogenicity and antibiotic resistance
- Phylogenetic algorithm(s) for clonal and sexually inherited markers, recombination, gene conversion, and horizontal gene transfer
- Capability to identify informative markers and their power to address specific forensic issues
- Better understanding of mutation rates and the effects of environment and host on these rates
- Discrimination and match criteria to quantitatively interpret results with confidence bounds

Components of a bioinformatic genetic toolbox for microbial forensics (continued)

- Capability to relate diversity to function
- Capability for comparative and functional genomics
- Contain or access curated (genetic marker) databases on pathogens and near neighbors and their background occurrence with epidemiological history, when available
- Data management with the capability to access and process large amounts of diverse genetic data and to communicate data rapidly with stringent informational security (i.e., fully functioning information interoperability)

A Complex Problem

- Law enforcement must be able to conduct traditional forensic examinations of evidence contaminated by hazardous CBRN materials
  - Requires special surety facilities
  - Requires special protocols
  - Requires specially trained Forensic Examiners

FBI Laboratory Hazardous Evidence Analysis Teams (HEAT)

- Forensic Examiners from various FBI Laboratory disciplines
- trained to conduct examinations of hazardous evidence

- Trace Evidence
- Latent Fingerprints
- Questioned Documents
- Firearm and Tool marks
- Special Photography
- Chemistry
- DNA
- Explosives
- Structural Design and Engineering
- Evidence Control

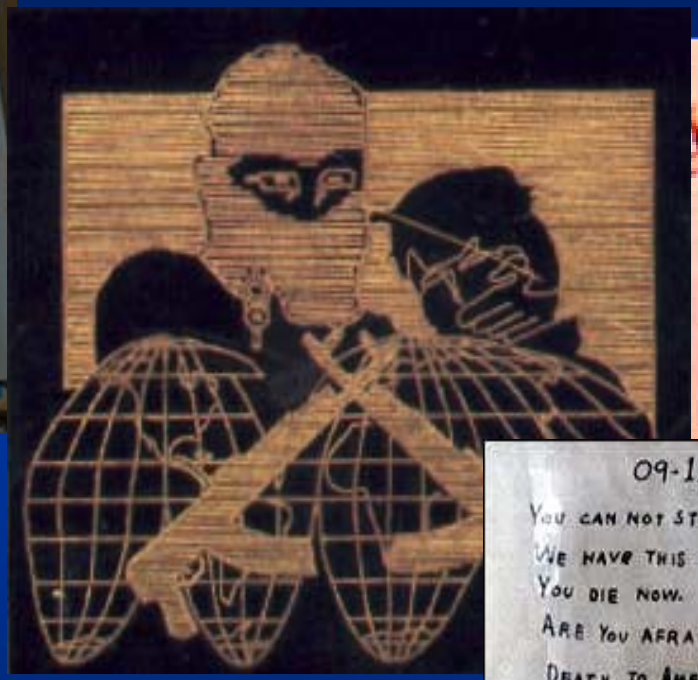
Elements of a Forensic Program

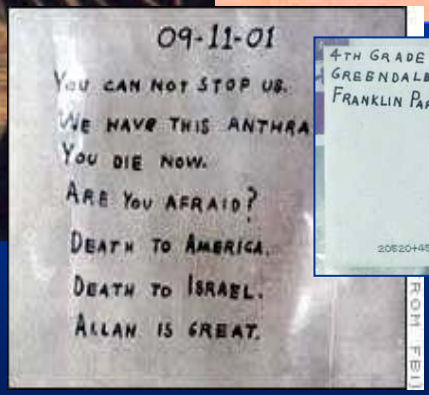
- Appropriate facilities (dedicated and properly configured)
- Validated protocols (sufficient detail)
- Accreditation or demonstrated compliance with established guidelines
- Scientifically defensible
- Legal admissibility

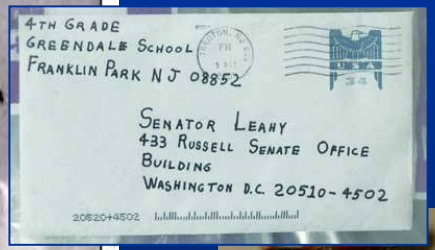
How to Improve Microbial Forensic Science Capabilities?

- Identify the experts
- Review the current (and past) “State of the Art” technologies
- Scientific working groups and establish guidelines
- Standards -- Standardization
- Define better and encourage validation and peer review of the science
- Share information and capabilities within the law enforcement community
- Foster partnerships

The Challenge for Microbial Forensics is: Source Exclusion, Association and "Attribution"







Five minutes before a party is NOT the time to learn how to dance

Snoopy to Woodstock