B69 Select Agent Microbial Forensic DNA Identification Techniques

Susan W. Jones, PhD, Richard P. Schoske, PhD, Stephen Francesconi, PhD, Lynn Cooper, PhD, Michael Dobson, PhD, and Robert Crawford, PhD, Armed Forces Institute of Pathology, 6825 16th Street, NW, Washington, DC 20306*

After attending this presentation, attendees will have knowledge about select agent microorganisms, DNA quantitation, and microbial DNA identification techniques.

This presentation will impact the forensic community and/or humanity by providing information about microbial forensic DNA identification techniques used in laboratories for the DNA identification of dangerous bacteria that are encountered in bioterrorist incidents.

The microbial select agents that have been historically been weaponized in foreign nations and that pose the greatest bioterrorist threat include *Bacillus anthracis spores*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei*, *Brucella abortus*, and *Clostridium botulinum toxin*, however, any microorganism or toxin can be used to create public chaos and fright in an act of biological terrorism or as a hoax. These microorganisms are cultured in a Biological Safety Level 3 (BSL3) biological containment laboratory, require special care in handling due to their pathogenicity, and are highly regulated in an effort to prevent further acts of bioterrorism.

Microbial class characteristics, such as gram stain reaction, cell morphology, biochemical metabolic profiles, presence of spores and toxin production are examined in microbial forensic analyses of organisms released in a terrorist incident or hoax. Knowledge of select agent (SA) microorganisms most likely to be used as biological weapons based on historical use, recent terrorist incidents involving SA microbes, and the need for more rapid identification of these microorganisms, has prompted the quest for informative DNA identification tests.

Microbial DNA identification tests require microbial strain population studies to assess the specificity, the informative value of the DNA assay for strain differentiation, and if source attribution of a strain is possible. Therefore, large repository of well characterized select agent micro-organisms for the production of microbial genomic DNA for PCR assay comparison studies is being grown in the laboratory of the AFIP. Information on microbiological characteristics of the select agent genus and species, genomic information, as well as any published information is also being collected. Sensitive, specific microbial PCR techniques, performed on specimens submitted for DNA identification, are based on detection of unique genes that are present in quantified microbial DNA and can be detected whether or not the microorganism is viable.

Various molecular biology techniques that have been used in the laboratory for microbial DNA identification include 16S ribosomal DNA sequencing, Amplified Fragment Length Polymorphism Polymerase Chain Reaction, Multi-locus Variable Number of Tandem Repeat Polymerase Chain Reaction, and Real-Time PCR amplification/detection of pathogen specific genes. The strategy of using combined microbiological and molecular biology techniques allows confident DNA identification, but not source attribution of select agent microorganisms.

Bioterrorism, Microbiology, DNA Identification

 ** Presenting Author*