B143 Development of a Microbial Forensics Real-Time PCR Assay for the Detection of Coxiella Burnetii

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After attending this presentation, attendees will have a basic understanding of *Coxiella burnetii* as it is used as a bioterrorism agent, how a real-time PCR assay for this bacterium is developed, and the positive impact that faster detection methods, such as real-time PCR, have on the microbial forensics community.

This presentation will impact the forensic community and/or humanity by providing knowledge about real-time PCR assay development for the detection of biothreat agents.

Coxiella burnetii, the causative agent of Q fever, is a gram negative, coccobacillus bacterium primarily found in cattle, goats, sheep, and other herd animals. Human contraction of this bacterium occurs from breathing aerosols containing amniotic fluid or fecal matter from herd animals. This pathogen is relatively resistant to heat, drying, and many common disinfectants. *C. burnetii* is on the list of select agents as determined by the Department of Health and Human Services and the United States Department of Agriculture because it is highly infectious and could be easily disseminated in aerosol form over a large area. Clinical symptomology of Q fever infection are similar to that of the flu including high fever, headache, muscle and joint pain, and coughing. Standard detection methods such as cell culture and antibody-based detection can be time consuming and labor intensive. The development of a rapid screening method is critical to ensure timely and accurate detection in a bioweapon event. Real-time Polymerase Chain Reaction (rtPCR) can be used as a quick and sensitive diagnostic tool and offers many advantages to standard detection methods.

The objective of this study was to design a sensitive and specific rtPCR assay for the detection of *C. burnetii* from clinical and environmental samples. The highly selective isocitrate dehydrogenase (*icd*) gene was chosen for the detection of *C. burnetii*. A comparison of 19 published *C. burnetii* strains was used to generate a consensus sequence to identify regions of similarity in the *icd* gene. PrimerExpress™ software was used to design a TaqMan® rtPCR primer and probe set, and the specificity of the assay design was verified using BLAST software. The FRET probe contained a 5`-reporter dye Cy5™ coupled with a 3'-Black Hole Quencher and the identified oligonucleotides primers were obtained from Integrated DNA Technologies (IDT; Coralville, IA).

This assay was found to be highly sensitive and specific for *C. burnetii*. An assay limit of detection of six gene copies/ìL was determined from serial dilutions of recombinant *C. burnetii* target DNA. The efficiency of this assay was calculated to be approximately 100%. Assay specificity was evaluated against a panel of pathogens including: genetically similar organisms, organisms that cause similar illness, common clinical and environmental microbes, and other select agent organisms. A specific rtPCR assay for *C. burnetii* would decrease the turn around time required for laboratory confirmation of suspected bioterrorism events and facilitate the proper treatment of exposed individuals. The primer and probe set will eventually be incorporated into a rtPCR multiplex being developed at the Biodefense Laboratory at Wadsworth Center (Albany, NY) for the detection of 13 different biothreat agents.

Microbial Forensics, Real-Time PCR, Coxiella Burnetii

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