B144 A Comparative Study of the ABI PRISM® 7500 to the ABI PRISM® 7500 Fast Real-Time PCR Platforms for the Detection of Biothreat Agents

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After attending this presentation, attendees will have a better understanding of how faster detection methods can aid in forensic investigations of bioterrorism.

This presentation will impact the forensic community and/or humanity by providing knowledge about two real-time PCR platforms that can be used to detect biothreat agents in clinical or environmental samples.

Bioterrorism involves the deliberate release of microorganisms or toxins derived from living organisms that cause illness or death in people, animals, or plants. Biological weapons have been used for centuries from the Assyrians poisoning the well water of their enemies with rye ergot to the Tartar army throwing plague ridden bodies over the walls of their adversaries. Today, with increased knowledge and technology, biological weapons pose a more serious threat than ever before. These weapons are inexpensive to manufacture, relatively easy to prepare and disseminate, and rapidly spread throughout a population. Biothreat agents are able to harm or kill many people in a short amount of time and can cause illnesses that are not recognized right away, delaying correct treatment and isolation of infected individuals. Because of these concerns, it is important to have rapid, sensitive, and robust methods of detection. Currently, a large number of detection methods are available to investigators including but not limited to viable organism culture, mass spectrometry, nucleic acid amplification/ detection (e.g., PCR), enzyme-linked immunosorbent assays, and suspension array technologies. Culture methods have been considered the gold standard for microbial identification for many years but it can be time consuming and is generally low throughput, making it unfavorable to use in a bioterrorism event. Real-time PCR (rtPCR) offers many advantages for detection, including high throughput, short assay time, low contamination risk, small genomic sample volume, excellent sensitivity and specificity, and potential to be multiplexed.

The objective of this study is to compare the Applied Biosystems (ABI, Foster City, CA) PRISM® 7500 rtPCR system to the ABI PRISM® 7500 Fast rtPCR system to show that the upgraded fast-block platform works as well with current validated protocols as the ABI PRISM® 7500 rt-PCR system. The TaqMan® rtPCR assay used for comparison in this study was a Wadsworth Biodefense multiplex assay for the detection of ricin toxin, *Fracisella tularensis*, *Yersinia pestis*, and *Brucella* species. In this study, the LightCycler® FastStart DNA Master *Hyb*Probe hot start reaction mix (Roche Applied Science, Indianapolis, IN) was used because this master mix was shown in previous studies to perform better than the ABI master mix when inhibitors were present in clinical or environmental samples.

The ABI PRISM® 7500 Fast rtPCR platform is similar to the 7500 platform; both support a 96-well plate format and have a five color detection system. The heating block in the ABI PRISM® 7500 Fast instrument has been modified, allowing it to heat and cool more rapidly than the ABI PRISM® 7500 model. The results of this study show that the multiplex assay run times were reduced from 100 minutes on the ABI PRISM® 7500 rtPCR platform to 45 minutes on the ABI PRISM® 7500 Fast rtPCR platform without losing sensitivity. In the advent of a bioterrorism attack, the use of a faster rtPCR system would increase the number of samples screened for potential biothreat agents thus decreasing the amount of time it would take health officials to administer the correct treatment to those exposed or infected.

Microbial Forensics, Real-Time PCR, Bioterrorism

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