B142 Multiplex PCR Detection of Three Class A Bioterrorism Agents for Use in a Miniature Ceramic PCR Device

Jason R. Besecker, BS, Korey Moeller, BS, Ken Cornell, PhD, and Greg Hampikian, PhD, Boise State University, 1910 University Drive, Boise, ID 83725*

After attending this presentation, attendees will be informed on the subject of biological warfare agents, and current research on the development of a multiplex PCR assay for the detection of three Class A select agents. This presentation will also discuss the development of a miniaturized PCR device constructed from novel materials for the use as a biosensor.

This presentation will impact the forensic community and/or humanity by demonstrating introducing a new PCR detection system that is capable of specifically amplifying three select agents with high sensitivity.

Throughout history biological organisms have been used for the purpose of warfare. It is known that some countries, such as Iraq and the former Soviet Union, have developed weaponized forms of biological warfare agents (BWA's) that have the potential to kill more people than nuclear or chemical attacks. It is estimated that ten grams of weaponized anthrax spores could result in the deaths of as many people as an attack using a ton of the nerve agent sarin.

Protecting the United States from biowarfare and bioterrorism requires detectors capable of identifying a collection of agents that might be used in a biological attack; methods currently used to identify the release of BWA's are inadequate. The goal of this project is to develop a multiplex PCR system capable of specifically and selectively amplifying target regions of three select bioterrorism agents. The developed multiplex assay is to be used in a PCR device constructed of a novel material, low temperature co-fired ceramic (LTCC). This device is being developed at Boise State University as collaboration between this laboratory (G.H.) and Dr. Amy Moll's laboratory in the College of Engineering.

Select agents chosen for use in the multiplex assay are *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis*; all of which are Category A select agents on the CDC's list of bioterrorism agents. Both high sensitivity and specificity are required of the multiplex PCR system to reduce the risk of false negative and false positive results. Primer sets for two different genes in each organism were selected to improve sensitivity and reliability of the assay. The majority of the selected gene targets are located on plasmids. Since plasmids generally occur in higher copy number than chromosomal DNA, sensitivity of the assay will be improved. Most of the chosen gene targets are virulence factors, which will help the assay discriminate pathogenic strains from non- virulent close relatives.

PCR primers used in the assay, which have previously been tested to specifically amplify regions of these species' DNA, were chosen from the literature. Working with Category A select agents requires a biosafety level 3 facility. Boise State University is biosafety level 2, so working with the actual organisms is not possible. To bypass this obstacle, DNA from non-virulent or attenuated strains of the bacteria were initially used in PCR reactions to obtain amplicons. Subsequently, the amplicons were ligated into plasmids then transformed into competent E. coli cells for replication. Plasmids were extracted from the

E. coli and concentration of extracted plasmid DNA was determined using a spectrophotometer. Ten fold serial dilutions were performed on the plasmids until one plasmid copy is present in one µl. These plasmid dilutions were used to initially optimize conditions for the multiplex PCR and test sensitivity of the assay to establish a detection limit for each individual primer pair. Specificity of the multiplex assay has been tested by performing PCR reactions on samples containing the recombinant plasmids and a myriad of microbial contaminants, including closely related species. Also, serial diluted plasmid DNA was tested in contaminated samples to determine if background bacteria interfere with the detection limit of the assay. When all conditions for the multiplex assay were optimized using the recombinant plasmids, the attenuated strains of the organisms were tested in the multiplex assay. Tests for specificity and sensitivity were carried out in the same manner as in the plasmid assays. After the multiplex assay has been optimized, and tested for sensitivity and specificity using attenuated strains of the organisms, PCR reactions will be tested in a device that is constructed of LTCC using samples of the actual class A select agents.

Bioterrorism, PCR, Detection

 ** Presenting Author*