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Sensitive and Rapid Biosensor and Assays for Detection of *Bacillus anthracis* Spores

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Background:

Creatv MicroTech is developing a multi-step biosensor for the detection of *Bacillus anthracis* spores combining immunoassay, culture and real-time PCR. The immunoassay results are reported here. This research covers three areas: antibody evaluation, assay development and instrument development.

Method:

Antibody affinity and specificity were evaluated using magnetic beads and plating. The instrument technology is based on the Integrating Waveguide Biosensor developed by the Naval Research Laboratory. This biosensor provides a lower limit of detection by reducing background noise, thus improving the signal-to-noise ratio. The instrument was constructed for the use of capillary tubes as the waveguide. Sandwich immunoassays were performed on the inner surface of the capillary tubes using Cy-5 fluorescent dyes. Two immunoassay methods were developed and evaluated.

Result:

The polyclonal antibody from Naval Medical Research Center (NMRC) had the highest affinity among the antibodies evaluated, but cross-reacted with other *Bacillus* species. The assay was able to detect <2000 spores.

Conclusions:

The high affinity of the NMRC antibody was critical for achieving high sensitivity in the multi-step biosensor. A subsequent real-time PCR step will be used to provide specificity, as described in a separate poster entitled "A Biosensor Platform for Genetic Identification and Quantitation of *Bacillus anthracis*." We expect to further increase detection sensitivity by optimizing the assay and using quantum dot fluorescent dyes.

Key Words: anthrax spore antibody evaluation, biosensor, anthrax detection