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A Biosensor Platform for Genetic Identification and Quantitation of *Bacillus anthracis*

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Background:
Real-time PCR assays for identification and quantitation of *Bacillus anthracis* were developed by Creatv MicroTech, Inc for detection of *B. anthracis* spores in environmental samples.

Method:
Three genetic markers were detected in the PCR reactions, utilizing DNA extracted from *B. anthracis* spores and vegetative cells. A chromosomal marker *rpoB* encoding the RNA polymerase β-subunit was used in real-time PCR to quantify genome copy numbers of *B. anthracis* spores and vegetative cells. Two plasmid markers, *pagA* and *capA*, which are involved in the biosynthesis of anthrax toxin and capsule, respectively, were used for the identification of virulent strains containing plasmids PXO1 and PXO2. In addition, an internal positive control (IPC) was also developed as a quality control of the PCR reaction and to detect potential PCR inhibitors in environmental samples. A standard curve was constructed for the quantitative real-time PCR.

Results:
There was strong linear inverse relationship (R^2=0.997) between threshold cycle (CT) and the $\log_{10}$ number of *rpoB* copies over 7 orders of magnitude. The detection limit was less than 10 copies of *B. anthracis*. Fifteen representative strains of *Bacillus* species were tested in this study. The results showed that the real-time PCR assays were specific to *B. anthracis* and did not cross-react with *B. thuringiensis* and *B. cereus*.

Conclusions:
By coupling this procedure with the immuno-capture and enrichment procedures in Creatv's biosensor system, the real-time PCR procedure established in this study provides high sensitivity and specificity for rapid identification and quantitation of *B. anthracis* in environmental samples.