Office of Technology Transfer

# Portable System for Microbial Sample Preparation and Bacillus Cereus Group Pathogen Analysis with Oligonucleotide Microarray

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We have developed a three-component system for microbial identification in decentralized laboratories that consists of (i) a universal syringe-operated silica minicolumn for successive DNA and RNA isolation, fractionation, fragmentation, fluorescent labeling, and removal of excess free label and short oligonucleotides; (ii) microarrays of immobilized oligonucleotide probes for rRNA identification; and (iii) a portable device for imaging the hybridization of fluorescently labeled RNA fragments with the arrays. The minicolumn was developed in two formats, manually- and centrifuge-operated, and it combines a guanidine thiocyanate method of nucleic acid isolation with a newly developed spore/cell disruption procedure and hydroxyl radical-based technique for fast (takes only minutes) DNA and RNA labeling and fragmentation.

For B. anthracis, B.cereus, B. thuringiensis, B. mycoides and B. pseudomicoides discrimination, B. cereus group microorganisms were re-classified on the basis of their 16S rRNA, 23S rRNA and gyrB gene sequences. Markers obtained were used for development of a discriminative oligonucleotide microarray. After bacterial sample hybridization, the overall fluorescence pattern is captured as a digital image with a 3.5-pound portable reader. The procedure of pathogen identification with the three-component system is rapid. It takes only 1h or 2h for identification of bacterial cells, or spores, respectively.

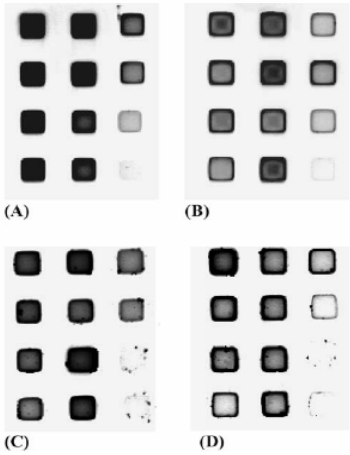


Fig. 6. Hybridization of labeled 16S rDNA produced by PCR amplification of bulk DNA from *B. cereus* T. (A) OP–Cu indirect protocol; (B) OP–Cu direct protocol; (C) Rapid direct OP–Cu protocol; (D) Rapid direct Fe–EDTA protocol.

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