B11 Evaluation of Commercial DNA Extraction Kits Using Bacterial Spores Associated With Problematic Matrices

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After attending this presentation, attendees will retain that commercially available kits can provide high quality DNA from problematic matrices, including soils, foods, drinks, and plants.

Although the kits tested are intended for microbial samples, this presentation will impact the forensic community and/or humanity by demonstrating how they can also be applied to situations in which the extraction of human DNA is required from problematic matrices.

Sensitive and specific PCR-based assays are available for detecting a variety of pathogenic microorganisms which may be used as bioterror agents; however, difficulties with these techniques may be encountered if the organisms are associated with materials such as soils, food and drink items, and plants. Because of inefficient separation of biological material from the surrounding matrix, suboptimal cell lysis, and co-purification of PCR-inhibitory substances along with nucleic acids, traditional laboratory methods for isolating DNA may prove ineffective for such samples. In cases where the release of a biological threat agent results in amounts of potentially hazardous microorganisms higher than those found in the environment, it is essential that high quality DNA can be extracted and used for PCR-based detection and identification.

Commercially available DNA extraction kits are designed to efficiently release nucleic acids from bacteria and other cell types typically found in the soil, as well as to remove or neutralize substances which may inhibit downstream applications such as PCR. In this study, several kits have been evaluated with respect to reproducibility and quality of the end product as obtained from a variety of problematic environmental matrices. Loam, clay, and sand samples were spiked with *Bacillus cereus* spores (a surrogate for *B. anthracis*), allowed to incubate for various times, and then subjected to DNA extraction using the kits. The various soil types provided differing starting amounts of PCR-inhibitory humic and fulvic acids, as well as different levels of physical attachment of the surrogate cells to the matrix, and therefore different types of challenges to the materials and reagents included in each kit. A variety of food and drink items as well as leaf surfaces were also tested as materials from which to extract bacterial DNA, and represent examples of other problematic matrices that may be encountered in forensic scenarios. Real-time PCR amplification of a 145 base pair region from the *B. cereus* genome was used to determine the quantity of *B. cereus* DNA obtained from the spiked samples, as well as the effectiveness of the various kits at procuring high quality DNA free of PCR inhibitors.

To optimize the kits for use in the forensic laboratory, the manufacturers' protocols were modified as necessary, including the addition of an exogenous reagent that has been shown to effectively remove PCR inhibitors. The results of this study will be made available to the forensics community so that researchers and investigators may choose appropriate methods for isolating nucleic acids from different types of biological material incorporated into problematic matrices.

DNA Extraction Kits, Bacterial Spores, Problematic Matrices

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