B64 Development of Improved DNA Extraction Method for Detection of Biological Threat Agents Collected on Environmental Swabs

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Attendees will learn an improved method for recovering and detecting DNA from biological threat agents collected on environmental swabs.

This presentation will impact the forensic community and/or humanity by demonstrating that sensitive detection can be achieved by using an integrated approach for sample processing that considers recovery of agent from the sampling matrix, release of DNA, counteracting environmental inhibitors, concentration of extract, and robust PCR conditions.

Investigations of crimes involving dangerous biological agents often require the use of swabs and wipes to recover the biological evidence. Current laboratory protocols for rapid extraction and detection of *Bacillus anthracis* (BA) from environmental swabs often rely on the recovery of DNA present on the external surface of spores. Furthermore, these methods generally do not include ways to counteract environmental PCR inhibitors except for dilution of the original DNA sample. As a result, these methods can suffer from poor sensitivity of detection. Additionally, such methods for extraction of spore DNA may not be appropriate for extraction of nucleic acids from other biological threat agents such as viruses. This study was conducted to determine whether the sensitivity of detection can be increased by incorporating steps for lysis, DNA purification, and optimized PCR conditions.

An extraction method was developed which consists of placing a swab head inoculated with microorganisms into a 2 ml O-ring screw-cap tube containing lysis buffer, glass beads and polyvinylpolypyrrolidone. The microorganisms were lysed by bead-beating and the DNA extracted and purified using the Qiagen DNeasy kit. To counteract the effect of environmental PCR inhibitors, molecular biology-grade bovine serum albumin (BSA; 200 ng/mL) was included in the PCR reaction.

In order to evaluate this method for extraction of nucleic acids from various types of microorganisms, real-time PCR assays were developed for the detection of DNA from BA (a Gram positive bacterium), *Yersinia pestis* (YP; a Gram negative bacterium), Vaccinia virus (VAC; an enveloped virus) and Adenovirus (ADV; an un-enveloped virus). Using this extraction method, the reliable lower limit (10/10 extraction replicates showing positive results in duplicate PCR reactions) of detection by realtime PCR is 100 colony forming units (cfu) of BA spores and 100 plaque forming units (pfu) of Vaccinia virus inoculated directly onto clean, sterile swabs. In order to mimic dirty or dusty environmental samples, swabs were coated with 10 mg of urban particulate matter (NIST SRM 1648) prior to inoculation with microorganisms. Real-time PCR detection following DNA extraction from these dirty samples resulted in reliable lower limits of detection of 500 cfu BA and 100 pfu VAC. Despite the apparent poorer detection limit of BA in dust-coated swabs, 100 cfu could be detected in a majority (95% of extraction replicates and 90% of PCR replicates) of the samples tested. Similar studies are underway to determine the limits of detection for YP and ADV.

In conclusion, the authors have developed a method for the efficient extraction and sensitive detection of BA and VAC DNA from environmental swabs. Future studies will focus on the recovery and detection of other organisms and development of extraction methods for other sampling matrices.

Microbial Forensics, DNA Extraction, Biological Threat Agents

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