B104 Decontamination of Bacterial Spores Without Affecting DNA Molecular Analysis

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After attending this presentation, attendees will realize that protocols can be used to kill bacteria without destroying the ability to molecularly analyze the DNA contained within the organism.

This presentation will impact the forensic community and/or humanity by giving attendees a validated procedure for decontamination without destroying the DNA. The value of this approach is to preserve evidence while allowing clean up in cases where bacteria may be present. In addition, if decontamination has taken place in a rogue laboratory, there may still be evidence that can be evaluated for its DNA signatures. The evidence should not be discarded in such cases.

Following the accidental or intentional release of pathogens, surfaces become contaminated and must be treated with a chemical agent to kill the organisms. In rogue laboratories, where pathogens are cultured for criminal or terrorist acts, the perpetrators may decontaminate surfaces and equipment used to grow the organisms in order to destroy evidence. Although the non-viable pathogens cannot be characterized by their growth characteristics following decontamination, the question remains whether the DNA inside the cells can still be utilized in assays for identification and attribution. Thus, there is a requirement to determine the effects of common decontamination procedures on the activity of DNA in downstream analyses. Research was performed to characterize the effect of several decontamination procedures on DNA as determined by PCR and restriction enzyme analysis. Bacterial spores of *Bacillus cereus*, a simulant for *B. anthracis*, were chosen for the studies, because these are very challenging organisms to kill and thus require rigorous decontamination procedures to effect a standard kill of 6-logs in concentration. Suspensions of organisms and dried deposits of the organism on various materials and surfaces of forensic relevance were examined. Manufacturer's suggested protocols, CDC (paraformaldehyde), and EPA (bleach) protocols were followed to kill the organisms. The materials examined included tile, wood, carpet, cubicle cloth, and painted dry wall. Real-time PCR and pulsed field gel electrophoresis (PFGE) were performed to evaluate the effects on both amplification and restriction digestion, respectively.

It was found that 10% bleach was effective in killing the spores at both pH 7 and pH 11, but the matrix on which the organisms were deposited significantly affected the ability to kill the spores. Actril (a mixture of peroxyacetic acid, hydrogen peroxide, and acetic acid), a commercial agent used in hospitals, was as efficient at killing spores as bleach. Actril worked well on all the surfaces and materials tested. DNA extracted from spores exposed to Actril can be amplified more efficiently and consistently than DNA extracted from spores exposed to bleach. PFGE did not reveal differences between the untreated control and Actril-treated spores. Paraformaldehyde (which is converted to formalin gas during the procedure) was very effective, but the DNA was minimally useful for molecular analyses. L-gel, a decontaminating agent reported to be capable of destroying both chemical and biological warfare agents, was not very effective on the spores when the agent was spread over the sample. DNA could be subsequently amplified from the recovered organisms treated with L-gel, but it could not be ruled out that this DNA was derived from cells that remained viable. Sterilox could kill the spores, but the efficacy of this agent was highly affected by the matrix on which the spores were deposited. The DNA was not damaged by Sterilox treatment of the spores. Actril and bleach are the most effective sporicidal agents, and Actril has the least effect on the molecular assays.

A validated protocol for the detection of bacteria collected from different surfaces that have been decontaminated by a variety of processes will be presented.

Decontamination, Nucleic Acid Amplification/Digestion, Pathogenic Microorganisms

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