A97 Demonstrating the Efficacy of Ethylene Oxide Sterilization for the Reduction of DNA in Plastics Used for DNA Extraction

Lisa M. Calandro, MPH, Ryo Hasegawa, BS, John Dektar, PhD, Liwei Qi, MS, Harmandeep Gill, PhD, and Jason Liu, PhD, Life Technologies, 850 Lincoln Centre Drive, Foster City, CA 94404*

The goal of this presentation is to describe the comparison of various sterilization methods for their ability to reduce the presence of amplifiable human DNA contamination. Additional studies also evaluated the effects of post-production sterilization on downstream DNA analysis.

This presentation will impact the forensic science community by providing information regarding options for decreasing the risk of DNA contamination, addressing a critical need as DNA typing methods become increasingly more sensitive.

Over the past several years, DNA analysis methods have become increasingly more sensitive with regard to the detection of very low quantities of DNA. Concurrently, laboratories have implemented automated processes to enable higher throughput and increased efficiencies within the laboratory. Many laboratories have moved away from manual extraction methods such as phenol: chloroform extraction in favor of automated bench top or high throughput systems. These systems are favored as they minimize the interaction between the analyst and the sample, reducing the risk of DNA contamination by the operator and the likelihood of sample switching errors. However, this trend has increased the burden on manufacturers of forensic products to ensure that collection devices and system consumables are free of extraneous DNA. This was highlighted in the recent "Phantom of Heilbronn" case in which a female profile, later attributed to contaminated swab collectors, was detected in evidence from numerous serious crimes throughout Germany. A joint publication issued by the ENFSI, SWGDAM and BSAG organizations (*Forensic Science International*: Genetics 4 (2010) 269–270) highlights the need for controls in the manufacture of consumables used for DNA analysis in order to minimize the introduction of human DNA. A number of measures can be introduced at the manufacturing site including manufacturing in a clean room environment, implementing extensive personal protective equipment (PPE) and automating the filling and assembly lines. Additional benefits may be achieved through post-packaging sterilization as a final measure of extraneous DNA removal. A series of studies to evaluate the efficacy of various sterilization methods including gamma irradiation, ultraviolet, electron beam and ethylene oxide (EtO) sterilization for the removal of DNA from plastics used for DNA extraction have been performed. Ethylene oxide is a widely accepted gas phase sterilization technique in the medical industry for the elimination of viable micro-organisms from medical devices and has recently been demonstrated to effectively minimize the presence of amplifiable DNA. In order to evaluate these sterilization methods applicable samples were spiked with extracted DNA and cellular material to mimic conditions of contamination. The spiked samples were provided to various sterilization vendors for treatment and then compared to untreated samples. The efficacy of DNA removal was evaluated using real-time PCR and STR-based detection methods. The studies demonstrated significant reductions in contaminating DNA from samples spiked with extracted DNA or cellular material using dual-cycle ethylene oxide treatment. Gamma irradiation, ultraviolet and electron beam treatment were less effective at reducing the presence of contaminating DNA. Further studies were performed to determine whether the ethylene oxide treatment would result in any deleterious effects on downstream sample processing for samples extracted with EtO-treated plastics. Treated and untreated plastics were used to extract a range of sample types. The extracts were subjected to downstream processing with real-time PCR and STR-based detection methods. Among the comparisons made for data generated from treated and untreated plastics were an evaluation of DNA recovery, profile quality, overall peak height, intra-color balance and artifacts. Ethylene oxide treatment was demonstrated to significantly reduce the risk of human DNA contamination without detrimentally affecting downstream results.

Contamination, Sterilization, DNA

