A196 Using the Autoclave for DNA Decontamination of Consumables for Use in Low Template DNA Testing

Michael A. Donley, MS, Dustin Foley, MS, and Mark Powell, MS, Harris County Medical Examiner's Office, 1885 Old Spanish Trail, Houston, TX 77054; and Lisa Gefrides, MS, Roger Kahn, PhD, Harris County Inistitute of Forensic Science, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will gain knowledge of different decontamination methods to remove consumable contamination, their application, and which method is the best approach to successful DNA decontamination.

This presentation will impact the forensic science community by providing information on three decontamination techniques and their effectiveness in a side-by-side comparison. This information will allow laboratories to make an informed decision when selecting a decontamination strategy with the intended result of minimizing consumable contamination in attendees' laboratories.

Technological advancements have greatly increased the sensitivity of DNA testing in recent years. Techniques for low template autosomal DNA testing focus on enhancing the amplification and the detection of the amplified product using capillary electrophoresis. Amplification is enhanced by increasing both the number of amplification cycles and the amount of polymerase in the reaction mix. Detection can be enhanced for low level samples by increasing injection times or by post-PCR treatment to allow more amplified product to enter the capillary.

As sensitivity increases so does the risk of detecting low levels of DNA from the laboratory environment during the testing process. Labs that perform extremely sensitive DNA testing, such as low template protocols (also known as LCN testing) and mitochondrial DNA testing, take great care to protect samples from contamination. For example, testing is generally performed in separate facilities from those used for routine autosomal casework. To combat low levels of exogenous DNA present in consumables or reagents, many laboratories UV irradiate consumables and reagents prior to use in casework. While UV irradiation is effective to some extent, it is not always able to remove all exogenous DNA present in a consumable item. Even after UV treatment, consumable contamination of casework samples may still be detected at a low rate. Recently, ethylene oxide (EO) treatment and autoclaving have been reported as alternatives to UV irradiation for treatment of consumables. Previous studies comparing autoclaving and UV irradiation of consumables for traditional STR typing have shown that autoclaving performs better than UV irradiation at eliminating exogenous DNA (Gefrides et al. 2010).[1]

The present study was undertaken to assess which method, UV, autoclaving, or EO, would perform best at removing contaminating DNA from consumables for low template testing. This study consisted of placing 50 µl, 25 µl, or 10 µl of blood or saliva on cotton swabs and 2 ml tubes and allowing them to dry overnight. The samples were then treated in the autoclave for one, two, or three hours. Samples were quantified using real-time PCR and a commercial kit that detected human and male DNA at the same time. Following quantification, samples were amplified at 31 cycles using a single-amplification inhibition-resistant amplification kit. The amplicons were then injected into a 3130xl genetic analyzer for 10 seconds.

None of the treated samples obtained a quantification value after three hours of autoclaving. Only three out of the 12 samples obtained a quantification value after two hours of autoclaving. The general trend is consistent with previous studies (Gefrides et al. 2010) where, after two hours of UV or autoclave treatment, no alleles were detected following 28-cycle amplification.[1] In those studies, however, after two hours of UV treatment (~7000 mJ/cm^2) alleles were still detectable with a DNA typing kit that uses 30 rather than 28 cycles of PCR amplification, suggesting that UV irradiation might not perform as well as autoclaving with increased numbers of amplification cycles.

Preliminary data for blood and saliva placed on cotton swabs indicate that a three hour autoclave treatment decontaminated consumables containing 10 - 50 µl of dried blood or saliva even after 31 amplification cycles. A two hour treatment was effective at decontaminating swabs with 10 – 50 µl of saliva or blood except for a single allele in one sample. After one hour of autoclave treatment, alleles were detected in all blood swabs while only one of the 50 µl saliva swab obtained alleles.

To complete this study, 50 µl, 25 µl, and 10 µl of blood or saliva samples on cotton swabs in 2 ml tubes will be treated with either two cycles of EO treatment or with one, two, or three hours of UV exposure in a crosslinker at a UV dose ranging from 3500 - 10,000 mJ/cm^2 (per Gefrides et al 2010). These samples will be subjected to low template amplification as well as mitochondrial DNA testing. Attendees will be presented with the findings of all studies and recommendations for the best methods for decontaminating consumables.

Reference:

1. L.A. Gefrides, M.C. Powell, M.A. Donley, R. Kahn. UV irradiation and autoclave treatment for elimination of contaminating DNA from laboratory consumable. FSI:Genetics 4 (2010) 89-94.

 ** Presenting Author*

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 ** Presenting Author*