A54 Evolving Strategies to Mitigate the PCR Inhibitory Compounds Found in Carpet Backing

Deanna M. Calabrese, Carrie A. Thomas*, and Michael S. Adamowicz, PhD*, Univ of New Haven, 300 Boston Post Rd, West Haven, CT 06516*

After attending this presentation, attendees will have a better understanding of the possible benefits of potential approaches to processing carpet samples for DNA analysis in order to avoid the effects of polymerase chain reaction (PCR) inhibitory compounds found in the backing layers of interior carpet.

This presentation will impact the forensic science community by providing experimental evidence indicating that the PCR inhibitory compounds in carpet backing are concentrated in different layers of the carpet. Results will also be presented that show that by only using a portion of the backing layer, the effects of the inhibitors can be mitigated or eliminated entirely. This study may suggest potential changes to extraction protocols that laboratories may implement in order to increase the quality of the DNA profiles of samples collected from interior carpeting.

A common feature of most residential homes, as well as many commercial and public structures, is carpeting. Evidentiary biological samples such as blood and semen are often collected from crime scenes in the form of stains soaked into this interior carpet. Highquality DNA profiles can be challenging to develop from these samples due to the presence of PCR inhibitors that interfere with the amplification process. In this study, 40 interior carpet samples were collected from different manufacturers and commercial vendors and assessed for PCR inhibition. The carpet was broken down into its four basic components: the fibers, the first backing layer, the binding/adhesive layer, and the second backing layer. Each component was examined individually and the backing layers were examined together in all combinations in order to identify if the PCR inhibitors were localized or were contained in more than one component of the carpet structure. None of the carpet fibers demonstrated any significant levels of PCR inhibition; however, 65% (26/40) of the intact carpet backing layers demonstrated significant to total inhibition of PCR product as measured with the Applied Biosystems Quantifiler® human DNA kit. When separated into individual layers, or in combinations of two out of the possible three layers, PCR inhibition was most often apparent with the first backing layer and the binding/adhesive layer. The second backing layer demonstrated little, if any, inhibitory effects on PCR. These results were further supported when the samples were amplified using the Promega PowerPlex® 16 HS short tandem repeat kit. Samples extracted from the first backing and the binding/adhesive layers demonstrated a reduced or absent STR signal, while samples extracted from the second backing layer were rarely affected. Data were collected showing that the PCR inhibiting compounds co-extract with the DNA; extracts of DNA-free carpet were not themselves inhibitory. Diluting the inhibited samples was effective at restoring signal in some instances, but not in all of them, indicating that a more efficient strategy to process samples would be to avoid the inhibitory agents to the greatest extent possible. Dilutions can also involve multiple trials, wasting sample that may already be in low quantity.

In summary, two of the three layers that constitute the backing layer of interior carpeting are indicated as containing the compounds responsible for the PCR inhibition observed when working with biological samples extracted from carpet. The carpet fibers and the second backing layer do not demonstrate significant levels of inhibition. While forensic DNA examiners could ideally avoid any PCR inhibition by only processing the fiber layer, in a sample of low quality and/or quantity, isolating every possible bit of DNA may be necessary to ensure the best possible profile. Additionally, the fiber layer is the part of the carpet most often cleaned in an attempt to destroy evidence, and therefore is not always available. In these cases, examiners will need to use the carpet backing; however, this study indicates that only the second backing layer should be used in order to avoid the PCR inhibitory effects of the two other layers.

DNA, Carpet, PCR Inhibitors

 ** Presenting Author*