A78 Optimizing Extraction Techniques for the Retrieval of DNA From Evidence Swabs

Michael S. Adamowicz, PhD, University of New Haven, Department of Forensic Science, 300 Boston Post Road, West Haven, CT 06516; Dominique M. Stasulli, and Emily M. Sobestanovich, University of New Haven, 300 Boston Post Road, West Haven, CT 06516; and Todd W. Bille, MS, Bureau of Alcohol Tobacco Firearms and Explosives, National Laboratory Center, 6000 Ammendale Road, Ammendale, MD 20705*

After attending this presentation, attendees will have a better understanding of the possible benefits of potential modifications to the QIAamp® DNA Investigator Kit protocol when used to extract DNA from cells collected on cotton swabs. The methods evaluated include a variety of simple modifications to the manufacturer's "Surface and Buccal Swab" protocol.

This presentation will impact the forensic science community by providing experimental evidence demonstrating that some alterations to the manufacturer's extraction protocol can yield enhanced recovery of DNA from cotton swabs, while others provided no increase in yield or show decreased yield as compared to the published kit protocol. This study may suggest potential changes to extraction protocols that laboratories may implement in order to increase the yield of their DNA samples collected on cotton swabs.

Samples for DNA analysis are often collected from a wide variety of objects using cotton tipped swabs. However, the question remains: are all of the collected cells being released from the cotton fibers of the swab? With the advent of increasingly sensitive STR kits, more and more samples are being submitted for DNA analysis which have small quantities of DNA present, degraded DNA present, or both. Samples such as firearms and other handled objects fall into this category and have become items commonly submitted to forensic DNA laboratories. When processing these types of samples the recovery of the maximum amount of available DNA becomes critical, potentially dictating whether or not a usable profile can be derived for a piece of evidence.

The QIAamp® DNA Investigator Kit is a rapid and effective extraction tool for forensic samples. It can be used to process a wide variety of evidentiary materials and requires relatively little handling of the samples as compared to a phenol/chloroform/isoamyl alcohol DNA extraction. Using the standard protocol as a baseline, the following parameters were altered: incubation time, incubation temperature, stationary incubation, physical disruption of the swab tip during incubation, and periodic re-suspension of the swab tip during incubation. Each of these conditions was performed as single variable experiments, as well as following up with extractions performed with a combination of conditions. All of the experiments were performed on cotton swabs which had either 10µl of liquid blood or 20µl of a buccal cell suspension dried onto them. All of the experiments were, at a minimum, performed in duplicate with both blood and buccal cells. Blank control swabs were also extracted for all conditions. Equivalent volumes of liquid blood and buccal cell suspension were also extracted in order to assess the retention of DNA on the cotton swabs. The concentration of DNA in each extract was quantified using the Applied Biosystems Quantifiler® Human DNA kit, and approximately one ng of extracted DNA was amplified with the Applied Biosystems AmpFl STR® Identifiler® Kit in order to assess the quality of the extracted DNA. Results indicate that up to 50% of the recoverable DNA may be retained on the cotton swab tip for both blood and buccal cells when using the standard extraction protocol. Stationary incubations performed poorly, with DNA yields falling significantly from those samples that were processed in a thermomixer. Incubation times of 18 or 24 hours showed no gain in the recovered quantities of DNA, and more often yielded less DNA that was of poorer quality. Some modest gains in yield were achieved with a three hour incubation coupled with an increase in temperature to 65°C with buccal cells. Physical disruption of the swabs was also no more effective than the standard protocol done with mixing. However, significant increases in DNA yields were observed using the swab re-suspension method for both blood and buccal cells, bringing the amount of recovered DNA close to the values observed for liquid samples.

In summary, while many alterations to the manufacturer's protocol were examined, only the swab re-suspension technique has shown significant gains in DNA yield to this point. These gains do appear to be substantial though, and further experiments will refine this technique to better enable forensic analysts to maximize the recovery of DNA from evidentiary swabs.

DNA, Cotton Swab, Extraction

 ** Presenting Author*