B85 An Improved Method for Extraction of DNA From Envelopes

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After attending this presentation, attendees will have a basic understanding of how to approach envelope DNA extractions in order to maximize DNA yield.

This presentation will impact the forensic science community by offering a more robust method for extracting DNA from historic envelopes with commonly found laboratory reagents.

To identify the best protocol for obtaining human DNA from envelopes, two sample collection methods were tested, involving both non-destructive and destructive methods, differing digestion solution volumes were evaluated, and two DNA extraction methods were compared.

At the Armed Forces DNA Identification Laboratory (AFDIL), envelopes can be used as alternative reference samples to help in the identification process. Currently at AFDIL, envelope DNA extractions are completed by: (1) using a steam bath to open the envelope seal; (2) swabbing the envelope seal to collect epithelial cells deposited when the envelope was licked; and, (3) extracting the swab using an organic extraction and purification method (PCIA, butanol, and ultra-4 column). Mitochondrial DNA (mtDNA) is targeted from envelope DNA extracts because it offers a direct comparison with mtDNA profiles from skeletal remains when nuclear DNA often yields incomplete profiles. Due to the sensitive nature of mtDNA analysis, the steam bath method can yield inconclusive results and introduce contamination from individuals who have previously handled the envelope.

This study developed a new method that maximizes the quantity of authentic DNA recovered from envelopes. To mimic casework conditions, mock envelopes containing a known mtDNA sequence in the seal of an envelope and a different known mtDNA sequence on the outside of the envelope were created for experimentation. These mock envelopes were used to evaluate how the following variables affected nuclear and mitochondrial DNA yields: (1) the amount of digestion buffer and proteinase K volumes; (2) the type of sample collection (steam bath-swabbing vs. cutting); and, (3) the type of extraction — organic vs. QIAamp® DNA Investigator Kit on the QIAcube®. Nuclear DNA yield was assessed by Quantifiler® Duo and mtDNA quality was assessed by mitochondrial DNA sequence data.

By changing the sample collection method to a cutting, the average amount of nuclear DNA recovered from each sample increased by nearly 40pg/µl, with a standard deviation of 9.2pg/µl. Increasing the amount of extraction buffer in the organic extraction process caused a similar increase in the amount of usable DNA. The average DNA recovered from those samples increased by roughly 15pg/µl, with a standard deviation of 8.4pg/µl. Attempts to use the QIAcube® did not prove fruitful, as the increase in recovered DNA was roughly 12pg/µl, while the improved organic extraction was nearly 60pg/µl.

The results indicate that using a cutting of the seal, rather than a swabbing of the seal, yields higher quantities of both nuclear and mtDNA after organic extraction. Secondly, the results show that increasing the volume of digestion buffer from 500µl to 1,200µl increases the quantity of DNA obtained. A comparison with the QIAamp® DNA Investigator extraction protocol performed on an automated QIAcube® instrument revealed that the organic extraction method resulted in higher quantities of DNA. No evidence of mixtures was observed despite the attempts to mimic casework conditions, most likely due to the high quality of the DNA within each mock envelope seal.

Continued evaluation of the method will incorporate heirloom envelopes similar to those encountered by the AFDIL casework sections. Additionally, the effect of Ultraviolet (UV) irradiation will be assessed to minimize the levels of exogenous DNA while not causing significant harm to the DNA contained within the seal.

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DNA Extraction, Envelope, Quantification