



H130 Manual Large Volume DNA Extraction and Purification From a Large Substrate

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Learning Overview: After attending this presentation, attendees will understand a new method to extract and purify DNA from a small number of epithelial cells present on a large substrate.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing a new method for DNA extraction. Current extraction methods are not able to fully submerge large substrates in a single sample tube, and the use of multiple small sample tubes can result in DNA loss and/or contamination. The method in this study takes these issues into account to improve upon the effectiveness of extracting DNA from large substrates.

A new Manual Large Volume (MLV) DNA extraction method has been developed to efficiently and effectively extract and purify DNA from a large substrate. The standard automated extraction protocol for samples with low-level DNA (referred to as standard extraction protocol) doesn't work for large substrates because of the small sample tube size. Currently, the method used at the Defense Forensic Science Center for large substrates, referred to as the multi-tube method, divides a substrate into smaller pieces and extracts each piece individually using the standard extraction protocol. To compare different methods of DNA extraction and purification in this study, a stock solution of saliva diluted with TE buffer at a fixed ratio was used. By using this solution for each method, a percent recovery can be calculated based on the standard extraction protocol. The multi-tube extraction method results in 60%–70% DNA recovery compared to the standard extraction protocol, which can likely be attributed to increased sample manipulation.

The MLV method allows for the entire substrate to be submerged in lysis buffer in a single sample tube, reducing sample manipulation and dilution/concentration steps. The MLV method was compared to the standard extraction protocol and multi-tube method to determine its effectiveness in extracting DNA from large substrates. All methods utilize a lysis buffer with proteinase K and a binding buffer. The standard extraction protocol and the multi-tube protocol use a bead-based automated extraction, while the MLV protocol uses a silica column to separate and purify DNA.

The MLV extraction method resulted in 80%–90% recovery compared to the standard extraction protocol when a diluted saliva sample was used. The MLV extraction method was also shown to be effective for extracting from a paper substrate and was able to yield pure DNA samples at 80%–100% recovery relative to the standard extraction from paper. To give a better understanding of its overall effectiveness and reproducibility, the MLV extraction method will be tested using other substrates that mimic case work samples.

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DNA Extraction, Large Volume, Manual Large Volume