B116 The Assessment of Variable Elution Volumes for Efficient Recovery of Low-Level DNA Samples From Robotic Extraction Platforms

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Learning Overview: After attending this presentation, attendees will better understand the complexities and challenges associated with the extraction, amplification, and analysis of low-level sample types such as touch DNA. Attendees will also learn techniques for manipulating the built-in feature of variable elution volumes with commonly employed robotic DNA extraction platforms to increase the quantity of DNA which can be recovered from low-level samples, subsequently increasing the probability of generating robust, highly-discriminatory Short Tandem Repeat (STR) profiles.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an efficient modern workflow solution for maximizing the amount of discriminating genetic information which can be produced from challenging low-level sample types such as touch DNA samples.

As the sensitivity of downstream processes in the DNA workflow continues to increase, case-working labs are seeing an increase in the number of samples being submitted for analysis with a concurrent decrease in the quality of samples being submitted. Many submissions are the result of property crimes or cold-cases, where there may be minimal traces of DNA amount of available sample remaining for retesting. To that end, it is imperative that front-end processes such as extraction can perform with increased stringency and efficiency. Recently, some of the major manufacturers of robotic DNA extraction platforms have released updated script cards allowing the end-user greater flexibility for elution volume used when extracting DNA samples. These options may prove beneficial in increasing the amount of genetic information which can be recovered from low-level challenging sample types, such as touch DNA.

The goal of the current research was to demonstrate a linear relationship between the elution volume applied on a robotic DNA extraction platform and total DNA yield in a sample. Using the Applied Biosystems® PrepFiler Express™ Forensic DNA Extraction Kit, a 1% serial blood dilution was extracted in triplicate on the Applied Biosystems® AutoMate Express™ DNA Extraction System. Following extraction, all samples were quantified using the Life Technologies Quantifiler® Trio quantification kit on an Applied Biosystems® 7500 Real-Time PCR System. Analysis of the quantitative data produced, as expected, a strongly negative correlation between increasing elution volume and decreasing sample concentration (r = -0.815, p = 0.0254). Calculation of total DNA yield resulted in a strongly positive correlation between increasing elution volume and increasing total DNA yield of the sample (r = 0.798, p = 0.0316). Average total DNA yield of the sample eluted at 20 µL (5.555 ng) was significantly lower than the average total DNA yield of the sample eluted at 250 µL (7.302 ng). A single-factor analysis of variance revealed a statistically significant difference in total DNA yield recovered at variable elution volumes (Fs = 4.517; df = 6, 20; p = 0.0094).

This study demonstrates that by increasing the elution volume applied to suspected low-level DNA samples, more total DNA can be recovered. Previously completed work has demonstrated the reliability of an in-house pre-amplification concentration protocol, utilizing an Eppendorf Vacufuge® plus vacuum concentrator. This data suggests that eluting low level DNA samples in higher elution volumes followed by pre-amplification concentration may allow for increased likelihoods of obtaining more complete, robust STR profiles as compared to eluting in lower volumes. Further work is currently being conducted to rigorously assess the quantitative and qualitative performance of low-level samples utilizing the proposed workflow with variations in buffer solution and robotic extraction platform selection.

Robotic DNA Extraction, Elution Volume, Quantitative Recovery