A133 Comparison of Collection Devices and Commonly Used Human Identification Kits for Forensic DNA Profiling of Soil-Inhibited Saliva-Skin Samples

Pamela J. Staton, PhD, Dishari Mukherjee, MBBS, Justin Godby, MSFS, and Tiffany Paugh, MS, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701*

After attending this presentation, attendees will be able to make an informed decision as to protocols best suited for casework involving soil contaminated saliva-skin samples as the main DNA source.

This presentation will impact the forensic science community by significantly reducing the waste of and processing time associated with the analysis of soil-inhibited saliva samples by identifying the combination of analytical procedures that will generate the best DNA profile. This presentation will help to solve cases involving violent crimes and sexual assault with a positive influence on the conversion of soil contaminated saliva-skin samples into a DNA profile. This study can also provide a basis for future studies to be conducted involving similarly inhibited samples.

Human body fluids such as blood and saliva are common biological materials frequently encountered in Forensic DNA Investigations. Saliva plays an important role as evidence in cases involving violent crimes through bitemarks and in addition through kissing and licking in sexual assault cases where neither semen nor blood is found, or where neither gives conclusive results. Saliva which has come in contact with intact skin remains stable and can be recovered at least 60 hours after deposition, but it is typically overlooked as a potential source of DNA as it is difficult to visualize. However, when bitemarks or bruises caused by kissing or sucking the skin are observed, saliva is likely to be present. Obtaining DNA profiles from saliva stains can be challenging because there is always the possibility of the evidence becoming contaminated with environmental inhibitors such as soil, which is both ubiquitous and abundant in nature. Soil contains powerful Polymerase Chain Reaction (PCR) inhibitors like humic acid and fulvic acid which can make obtaining a DNA profile difficult. Data from the U.K.'s National DNA Database (NDNADB, 2006) shows that saliva as a DNA source constitutes 33.3% of total samples recovered, making it statistically the largest source of DNA recovered from crime scenes. Blood, however, constitutes only 17.4% of total samples recovered. Of the 33.3% total saliva samples, only 43.1% generated DNA profiles suitable for loading on the NDNADB, which is in vast contrast to 92.8% of the 17.4% total blood samples that generated DNA profiles suitable for loading on the NDNADB. An optimum procedure is therefore required to maximize the probability of DNA recovery and genotyping from saliva samples. The goal of this study was to establish the best methodologies for collecting and profiling of soil contaminated saliva stains on skin using commercially available kits and supplies common in Forensic DNA laboratories. Saliva obtained from male donors was deposited on a measured area of a female volunteer's skin surface, keeping with the amount of saliva generally associated with human bite marks (250- 300µL). The testing samples were collected after allowing the saliva stain to properly dry. Two collection devices were used: polyester tipped sterile swabs (FisherBrand) and sample collection popules (Puritan). The double swab technique (wet and dry swabs, respectively) was used for collecting saliva samples with the swabs to maximize cell collection. Varying concentrations of soil were added to a portion of the saliva-skin samples after collection. Two magnetic particle based DNA extraction kits: Promega's DNA IQ™ and Applied Biosystem's (AB) Prepfiler™ were compared on the basis of their performance in DNA recovery. Quantification was performed using AB Quantifiler® Duo DNA Quantification Kit on the AB 7500 Sequence Detection System v1.2.3. PCR amplification was conducted with two multiplex STR systems: AB's and Promega's PowerPlex® 16 System, on the AB GeneAmp®

PCR System 9700. The amplified products were separated on the AB 3130xl Genetic Analyzer. The data generated was analyzed using AB's GeneMapper® ID Software v3.2.1. Sample peak height, allelic dropout, and artifacts such as pull-up were taken into consideration when making a determination of the best set of kits for generating DNA profiles of the aforementioned samples. Mixed profiles were not observed across most samples with only some profiles showing a few alleles from the female volunteer. This could be a result of DNA shedder variability. The project is ongoing, but statistical analysis between swabs and popules thus far show no significant difference between the two collection devices. Preliminary testing based on peak heights revealed that PowerPlex® 16 outperformed Identifiler® while DNA IQ™ proved to be better than Prepfiler™ on saliva-skin samples with soil. Additional testing and analysis will be required to make a determination of the ideal combination of kits for maximizing DNA recovery and generating reportable genetic profiles from said sample types. This study was conducted after IRB approval for human research was obtained.

Soil, Saliva, DNA Profiling

 ** Presenting Author*