

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

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ABSTRACT

Human body fluids such as blood and saliva are common biological materials encountered in forensic DNA investigations. In sexual assault cases, in which neither semen nor blood are found or give conclusive results, saliva may be utilized as forensic DNA evidence. While saliva may be deposited on skin via kissing, licking, sucking, or biting, obtaining DNA profiles from these areas can be challenging due to the presence of contaminants. One such contaminant is soil which is both ubiquitous and abundant in nature. Its constituents, such as humic acid and fulvic acid, are known to inhibit Polymerase Chain Reaction (PCR) by interfering with *Taq* Polymerase activity (7). Analytical procedure optimization can overcome this inhibition and make obtaining a DNA profile more efficient.

INTRODUCTION

This study was performed to establish the best methodologies for collecting and profiling soil contaminated saliva stains on skin using commercially available kits and supplies commonly used in Forensic DNA laboratories.

MATERIALS AND METHODS

Twenty ml of saliva was collected from a male volunteer, aliquotted, and stored at -20°C until use (8). Two hundred and fifty µL of saliva was applied to pre-measured test areas on the skin of a female volunteer and allowed to air dry for 10 minutes (3), see Figure 1. Prior to extraction, soil was added to the lysis buffer used in the extraction of collected swabs as seen in Table 1. The following flow chart depicts the comparison scheme utilized.

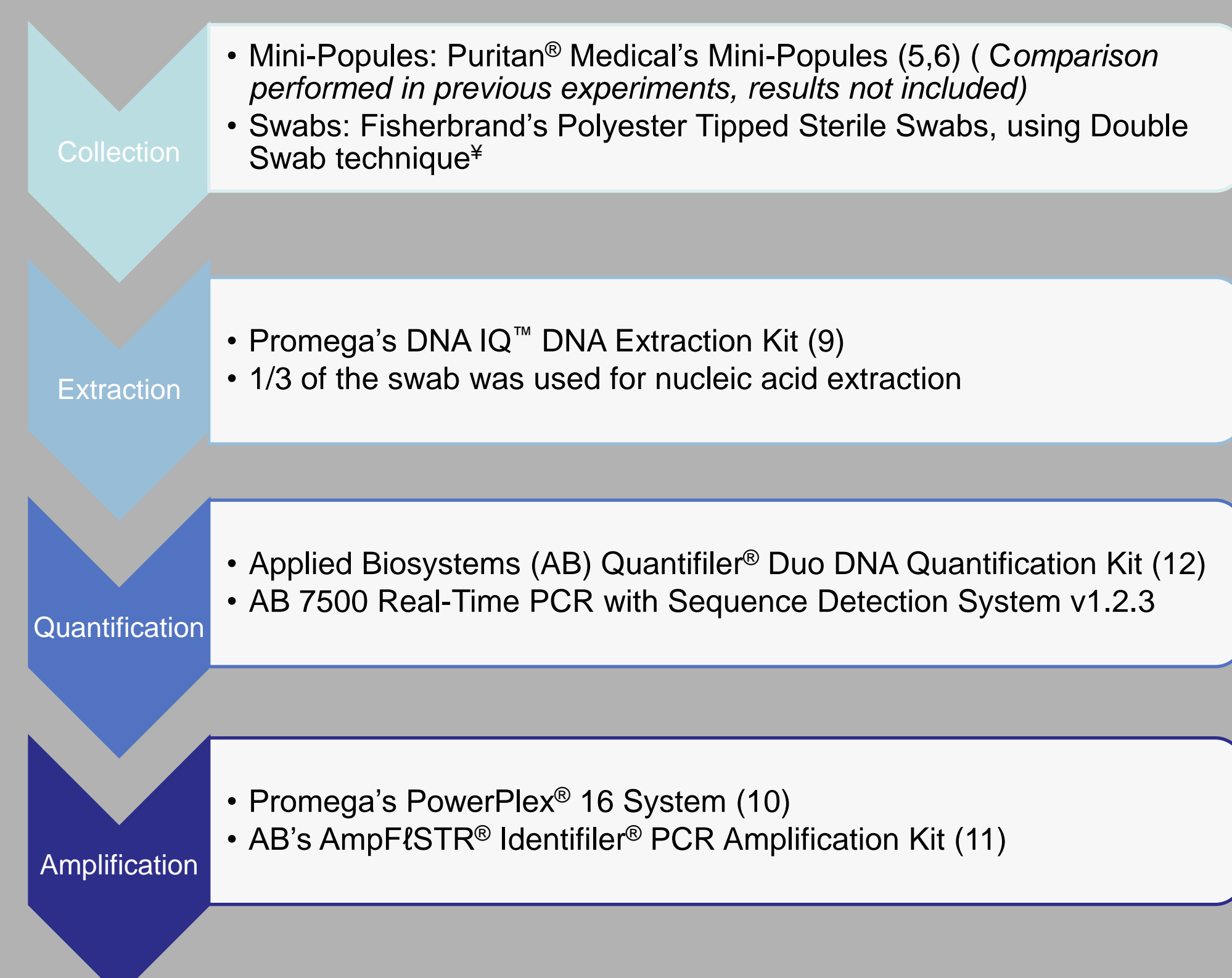


Figure 1. Collection of sample using Double Swab technique

Lysis Buffer for Initial Incubation		
Sample Name	Soil Quantity (mg)	DNA IQ™ Lysis Buffer (µL)
C0A+ B	0	400
C1A+ B	10	400
C2A+ B	50	400
C3A+ B	100	500
C4A+ B	250	500
C5A+ B	500	650
C6A+ B	1000	1050

A,B Samples processed with the same soil concentration.

Table 1. Soil Spiked Lysis Buffer for Initial Incubation

Capillary Electrophoresis was performed on the AB 3130xl Genetic Analyzer. Data analysis was performed using AB's GeneMapper® ID Software v3.2.1.

RESULTS

Table 2. Identifiler® and PowerPlex® 16 peak heights

Identifiler® vs. PowerPlex® 16 1.5ng Amplification Load		
Sample Name	Identifiler® Average Peak Height (RFU)	PowerPlex® 16 Average Peak Height (RFU)
IQC0A	572	470
IQC0B	815	1461
IQC1A	694	556
IQC1B	767	1032
IQC2A	1225	1843
IQC2B	NA	684
IQC3B	140	196
Average Peak Height:	702	892
2-Tail Confidence Level:		49.58%(Not Significant)

Table 3. Identifiler® amplified samples with significantly higher peak heights as compared to PowerPlex® 16

Identifiler® and PowerPlex® 16 Amplification Volume vs. Peak Height			
Swab Sample	Identifiler® 10µL	PowerPlex® 16 10µL	PowerPlex® 16 19.2µL
IQC0A	2060	1251	1623
IQC0B	4115	1432	1975
IQC1A	2243	1218	1545
IQC1B	1946	1041	1682
IQC2A	5427	2323	1008
IQC2B	1154	473	557
IQC3A	NA	345	362
Average Peak Height:	2824	1164	1336
2-Tailed Confidence Level:	97.10%(Significant)		95.60%(Significant)

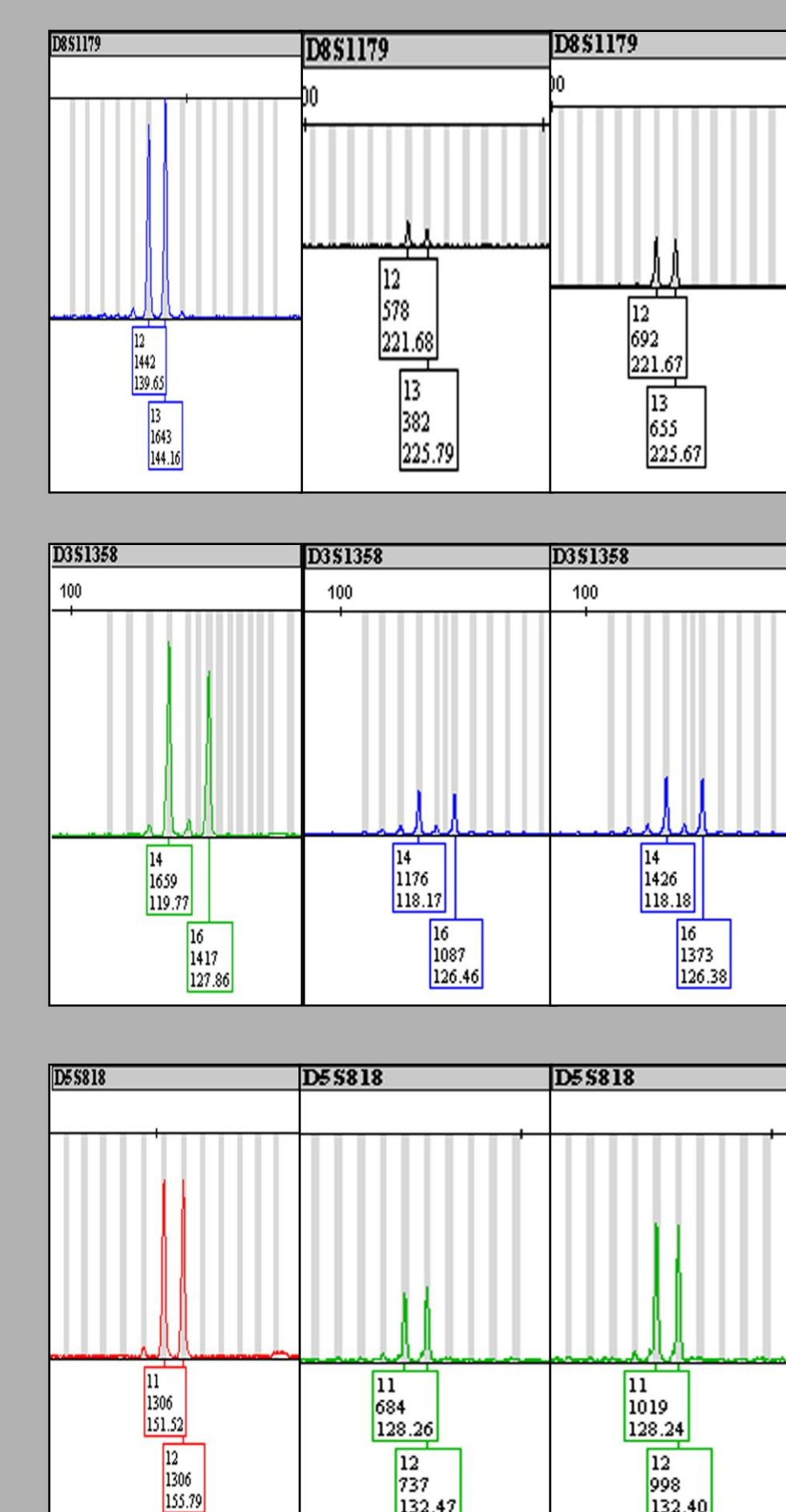


Figure 2. Higher peak height at loci with Identifiler® compared to PowerPlex® 16. D8S1179- Top, D3S1358- Middle, D5S818- Bottom

DISCUSSION

Previous experiments indicated no significant difference in peak heights with the use of mini-popules and polyester-tipped swabs as saliva collection devices. A comparison between the two is shown in Table 4. Due to the higher cost of mini-popules, the easy availability of swabs in Forensic DNA Laboratories, and the tailoring of extraction methods to swabs as the collection device for this comparison study. Data generated in this study depicted minimal mixed profiles attributable to epithelial cells collected from the female subject. This could be ascribed to DNA shedder variability. All samples experienced complete inhibition with the addition of ≥250 mg of soil. The remaining samples produced full profiles when spiked with ≤100 mg of soil. Exceptions are illustrated in Table 5. With the addition ≤100 mg of soil, Identifiler® amplified samples produced more full profiles as compared to PowerPlex® 16 amplicons.

Identifiler® data also exhibited higher average peak heights for samples overall as shown by 2-tailed t-tests, Tables 2 and 3. Identifiler® and PowerPlex® 16 peak height variation can be seen in Figure 2.

Table 4. Mini-Popules vs. Swabs as collection devices

Comparison Between Mini-Popules and Swabs	
Mini-Popules	Swabs
Isopropanol used as solvent	MBG water used as solvent
Solvent included	Not included
Detachable foam head	Head not detachable
Easier to cut into equal pieces	Difficult to cut
Air drying not required	Air drying required ≥ 30 minutes
Residual glue on foam head	No residual glue
\$364.34/ Case of 500 (Puritan® Medical)	\$322.74/Case of 1000 (Fisher Scientific)

Table 5. Identifiler® showing less number of soil inhibited samples

Sample Inhibition with ≤100 mg of soil (DNA IQ™ Extraction)		
Target DNA/Extract Volume	Amplification Kit	Inhibited Samples
1.5ng	Identifiler®	C2B, C3A, C3B (Partial)
1.5ng	PowerPlex® 16	C3A, C0A (Partial), C2B(Partial), C3B Partial)
10µL	Identifiler®	C3A, C3B*
10µL	PowerPlex® 16	C3A, C2B (Partial), C3B*
19.2µL	PowerPlex® 16	C3A, C2A (Partial), C2B (Partial), C3B*

A,B Samples processed with the same soil concentration. *Not Applicable

CONCLUSION

Based on the findings of this research, the methodology that is the best for profiling of male saliva on female skin, using kits and supplies commonly used in Forensic DNA labs, will be sample collection with polyester-tipped swabs, extraction using Promega's DNA IQ™ DNA Extraction kit and amplification with AB AmpFSTR® Identifiler® PCR Amplification Kit.

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*Double Swab Technique

A wet swab is prepared by dipping a swab into sterile, molecular biology grade water. The target surface is then swabbed for 15 s using medium pressure and circular movement. This is immediately followed by swabbing with a dry swab to collect the residual moisture left by the wet swab. The swabs are rotated along their long axis allowing every side of the swabs to come into contact with the target surface. The wet swab re-hydrates dried epithelial cells and leukocytes present in the saliva stain while the dry swab picks up these cells along with the residual moisture. The wet and the dry swabs are air-dried for >30 minutes and then pooled together for DNA extraction (1, 2).

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