Evaluation of a One-Step DNA Extraction Method for “touch” Samples

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INTRODUCTION

Due to advances in DNA typing technologies, trace amounts of DNA have been successfully genotyped from a variety of substrates, including weapons, gloves, and drinking containers (1-5). However, DNA extraction methods may not be efficient at retrieving adequate amounts of high quality DNA from “touch” and other low template samples for successful DNA profiling. Therefore, it is important to ensure that the initial collection and DNA purification methods are as efficient as possible in order to obtain the maximum amount of template DNA from each sample.

Many studies have investigated the comparative success of using various swabs versus tape-lifts when sampling touch DNA on evidence samples (6 – 8). Here, we evaluate swabbing versus tape lift collection using a Self Adhesive Security Seal Sticker® (Digifirma S.A) in conjunction with a new DNA purification method.

The Fingerprint DNA Finder (FDF®; nexttec™, Germany) is the fastest and easiest commercial DNA extraction system available using a single buffer and a one-step DNA purification based on the reversal of the silica principle. Proteins, detergents and other PCR inhibitors are bound to the surface of a special absorbent, while the nucleic acids pass through the column and retained in solution (Fig. 1). This approach may avoid DNA being retained in the column as has been reported when using bind-wash-elute methods (9,10).

In this study, three DNA extraction kits (FDF® kit, a modified version of the FDF® kit (with 50% reduced sorbent), and the QIAamp DNA Investigator kit) were used to evaluate DNA collection and extraction protocols for low template DNA “touch” samples.

MATERIALS AND METHODS

Sample Collection: Dice were used to simulate a smooth, nonporous surface, and lengths of rope were used for a rough, more porous surface for “touched” objects. Ten volunteers each deposited skin cells on sterilized dice (N=3 per volunteer) and rope (N=6) by rubbing all three dice, or all six rope samples between both hands for 3 min. (Fig. 2). All the protocols used in this study were approved by the IRB of Sam Houston State University.

DNA was collected from all sides of the dice using swabs (Bode SecurSwab™ DUO-V). FDF® Lysis buffer (30 µL and 20 µL) was applied to the head of the swab prior to DNA collection from the dice testing the original and reduced FDF® kits (nexttec™) respectively. For dice swabbed for extraction with the Investigator kit, 30 µL of 2% SDS was added to the tip of the swab prior to collection. Two sample collection methods were used to recover touch DNA from rope for comparison: 1) direct swabbing and 2) tape lift + swabbing (of tape).

For the tape lift method, each rope sample was placed length-wise in the middle of the adhesive side of the Self Adhesive Security Seal Sticker® (Digifirma S.A) and then the tapes was pressed together around the rope. The entire adhesive side of the tape was then swabbed with a single swab pre-wet with 30 µL or 20 µL of FDF® lysis buffer for the original and reduced FDF kits respectively, and 30 µL of 2% SDS for the Investigator kit.

RESULTS

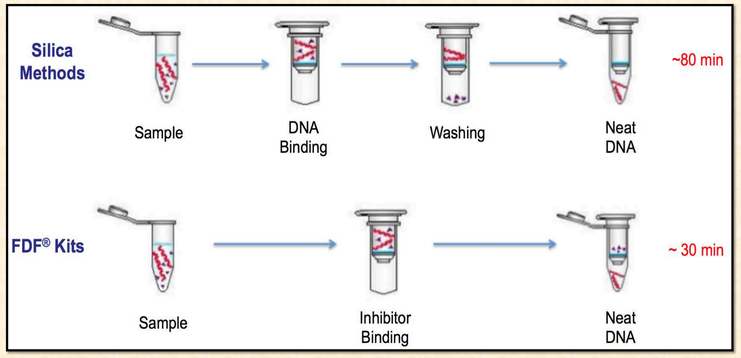

Figure 1: Silica-based and FDF® DNA extraction method. Proteins, detergents and low molecular weight components are bound to the sorbent and DNA is washed through the column.

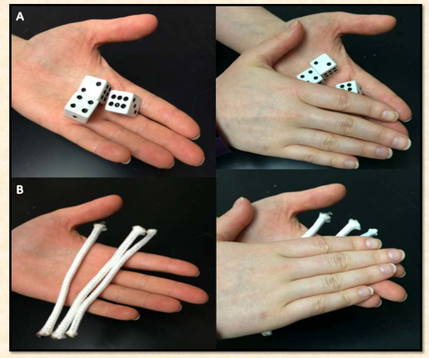

Figure 2: Controlled deposition of DNA onto A) dice and B) rope (N = 10 Individuals).

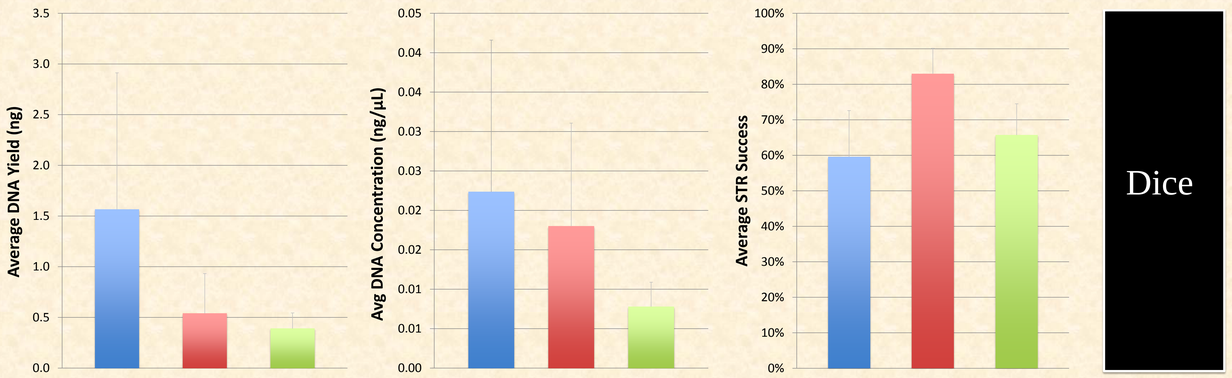

Figure 3: Average DNA yield, concentration of extracts (ng/µL) and STR success from dice swabbed and extracted using the DNA Investigator kit, the reduced FDF kit and the original FDF® kit.

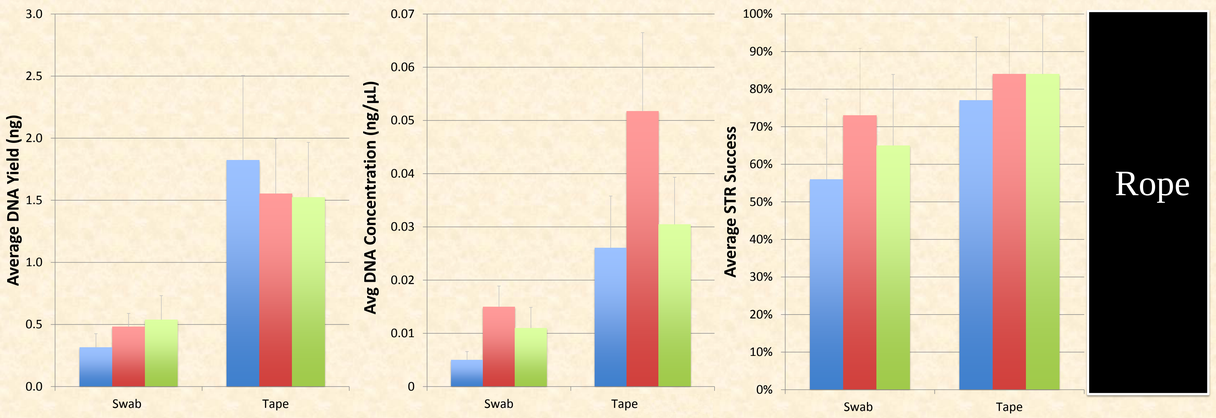

Figure 4: Average DNA yield, concentration of extracts (ng/µL) and STR success from rope using the DNA Investigator kit, the reduced FDF kit and the original FDF® kit collected via swabbing and a tape lift + swab (Self Adhesive Security Seal Sticker®) method.

MATERIALS AND METHODS

DNA Extraction: Swabs extracted with the original FDF® kit and DNA Investigator kits were processed as recommended. For swabs processed with the new (reduced) FDF® kit, half volumes of lysis buffer were used.

DNA Quantification: The amount of DNA per sample was determined using the QuantiFiler™ Human DNA Quantification Kit on a 7500 thermal cycler (Thermo Fisher Scientific)..

Genotyping and Data Analysis: All samples were genotyped using the GlobalFiler® PCR amplification kit (Thermo Fisher Scientific). Amplified products were resolved on a 3500 Genetic Analyzer with a 36cm capillary array and POP-4™ polymer, injected for 10 s at 3 kV and run at 15 kV.

CONCLUSIONS

Results of this study demonstrate that the FDF® kits are capable of extracting high quantity and quality DNA from “touch” evidence, using both swab and tape lift + swab methods.

- The FDF® kits purify high quantity and quality DNA from “touch” DNA faster (20 min versus 70 min) than silica-based methods and with much less sample handling.
- More than three times the amount of DNA was recovered from rough surfaces (rope) using the tape lift + swab method compared to swabbing (regardless of DNA extraction method used).
- The DNA Investigator kit produced the highest DNA yields. However, both FDF® formats produced more concentrated DNA extracts and higher STR success rates for most samples compared to the Investigator kit.
- Overall, the FDF® reduced format (50% less sorbent) performed the best in this study, generating the most concentrated DNA extracts and most complete STR profiles from “touch” samples.

ACKNOWLEDGEMENTS

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