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Increased recovery of touch DNA evidence using FTA paper compared to conventional collection methods

Irina A. Kirgiz [a], Cassandra Calloway [a, b, *]

[a] Forensic Science Graduate Program, University of California, Davis, 1909 Galileo Court, Suite B., Davis, CA 95618, USA
[b] Children's Hospital Oakland Research Institute, Oakland, CA, USA

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ABSTRACT

Tape lifting and FTA paper scraping methods were directly compared to traditional double swabbing for collecting touch DNA from car steering wheels (n = 70 cars). Touch DNA was collected from the left or right side of each steering wheel (randomized) using two sterile cotton swabs, while the other side was sampled using water-soluble tape or FTA paper cards. DNA was extracted and quantified in duplicate using qPCR. Quantifiable amounts of DNA were detected for 100% of the samples (n = 140) collected independent of the method. However, the DNA collection yield was dependent on the collection method. A statistically significant difference in DNA yield was observed between FTA scraping and double swabbing methods ($p = 0.0051$), with FTA paper collecting a two-fold higher amount. Statistical analysis showed no significant difference in DNA yields between the double swabbing and tape lifting techniques ($p = 0.21$).

Based on the DNA concentration required for 1 ng input, 47% of the samples collected using FTA paper would be expected to yield a short tandem repeat (STR) profile compared to 30% and 23% using double swabbing or tape, respectively. Further, 55% and 77% of the samples collected using double swabbing or tape, respectively, did not yield a high enough DNA concentration for the 0.5 ng of DNA input recommended for conventional STR kits and would be expected to result in a partial or no profile compared to 35% of the samples collected using FTA paper. STR analysis was conducted for a subset of the higher concentrated samples to confirm that the DNA collected from the steering wheel was from the driver. 32 samples were selected with DNA amounts of at least 1 ng total DNA (100 pg/μl when concentrated if required). A mixed STR profile was observed for 26 samples (88%) and the last driver was the major DNA contributor for 29 samples (94%). For one sample, the last driver was the minor DNA contributor. A full STR profile of the last driver was observed for 21 samples (69%) and a partial profile was observed for nine samples (25%); STR analysis failed for two samples collected using tape (6%).

In conclusion, we show that the FTA paper scraping method has the potential to collect higher DNA yields from touch DNA evidence deposited on non-porous surfaces often encountered in criminal cases compared to conventional methods.

1. Introduction

Each year approximately 800 thousand vehicles are stolen in the United States[1] and an estimated 4.9 million vehicles are stolen worldwide.[2] Many of the stolen vehicles are used to transport illegal substances or are involved in other types of crimes.[1] In many cases, touch DNA collected from the steering wheel is the only evidence that can link a perpetrator to the crime (*e.g.* most recent driver to the carjacking).[3]

Forensic analysis of touch DNA evidence was first described in 1997 by Van Oorschot and Jones (DNA fingerprints from fingerprints).[4] Since then, the topic of touch DNA has become of particular interest to researchers in the field of Forensic Science. Now this type of evidence is frequently used in criminal cases worldwide and the number of cases solved solely by virtue of touch DNA has grown dramatically.[5] As of today, a number of research teams have tried to standardize the touch DNA collection protocol.[3] Despite their effort,

* Corresponding author. Forensic Science Graduate Program, University of California, Davis, 1909 Galileo Court, Suite B., Davis, CA 95618, USA.
E-mail addresses: i_ilyenko@yahoo.com (I.A. Kirgiz), scalloway@chori.org (C. Calloway).

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there is a need for further improvements, since collection methods optimized for analysis of high copy number DNA evidence are not always effective for low copy DNA specimens.[3] Touch DNA often yields only a partial profile that is frequently attributed to a low shedding status of a donor.[6] However, low DNA yields can also be explained by ineffective collection methods that leave behind a portion of the deposited DNA.[7] Not all techniques that are used for blood, sperm and saliva DNA collection are effective for collecting touch DNA. DNA transferred to an object is usually present in smaller quantities at the crime scene when compared to the bodily fluids DNA.[3] Touch DNA is also invisible to the naked eye and often a forensic scientist can only approximate its location on the surface of an object.[3]

The most widely used method for collecting touch DNA evidence from surfaces uses cotton swabs and a double swabbing technique.[6,8–10] Tape lifting using water soluble tape is an alternative method that is used to collect touch DNA evidence.[11,12] A scraping method using Whatman® FTA® cards[13] is a novel approach which was used in one case study to collect touch DNA from the surface of a steering wheel.[14] Limited research has been published on the comparison of different touch DNA collection methods.[7,12,15]

The goal of our research was to directly compare double swabbing to tape lifting and FTA paper scraping methods for collecting touch DNA deposited on steering wheels of vehicles under circumstances that closely resemble a real life situation. The steering wheel is an ideal surface for touch DNA collection as it is relatively smooth and prone to a long contact with the driver's hands. In order to replicate a real life situation, we performed a blinded study with little or no information about the drivers, their shedding status, hygiene routine, and driving schedule. In order to maximize DNA recovery, DNA was collected over the entire area of each half of the steering wheel.

2. Materials and methods

2.1. DNA collection

This study compared three different DNA collection techniques of touch DNA from steering wheels: double swabbing, tape lifting and FTA paper scraping. A total of 70 cars were selected to participate in this study. All of the selected cars were in fully operational conditions; all cars were driven for 2–60 min on the day of the touch DNA collection. The steering wheels of the cars were selected for hard plastic, relative cleanliness and absence of wheel covers.

The study was approved by the Institutional Review Board (IRB) at the University of California, Davis. Car owners were asked to provide a reference cheek swab and answer a questionnaire. 35 cars were selected to compare the double swabbing method to tape lifting method, and another 35 cars were selected for double swabbing vs. FTA paper scraping method comparison. They were analyzed in the following way: one half of a steering wheel was sampled using a double swabbing technique, while the other side of a steering wheel was sampled using either a tape lifting technique (Fig. 1A) or the FTA paper scraping technique (Fig. 1B). We randomly alternated sides in order to minimize the possibility that the difference in DNA yield was due to a difference in shedding status between the left and right hands or bias in driving hand.

The study was divided into two parts for a direct comparison to the double swabbing technique: double swabbing vs. tape lifting, and double swabbing vs. FTA paper scraping. Fisherbrand® small 6 inch single headed sterile cotton-tipped swabs (Cat No. 23-400-115) were used for double swabbing procedure. The standard double swabbing technique[8] was used with the following modification. Both swabs were moistened with three drops of de-ionized water before the sample collection.[16] Due to a relatively large surface area of a steering wheel the moisture left by the first swab dried out before we were able to apply the second swab.[12] For the FTA paper scraping method, four drops of de-ionized water were applied to a 3.2 cm by 3.9 cm cut portion of a Whatman WB120205 FTA Classic Card before the sample collection. For tape collection, a 6 cm piece of 3 M(™) Water-Soluble Wave Solder Tape 5414 used to collect DNA from one side of the steering wheel.

2.2. DNA extraction and analysis

Swabs and FTA cards were dried for at least 1 h in a drying box. DNA was eluted from the cotton swabs following the procedure described in “DNA Purification from Buccal Swabs”. The Whatman FTA card was cut into small pieces and placed into two extraction tubes. The DNA was eluted from the FTA paper following manufacturer's procedure for “Isolation of Total DNA from FTA and Guthrie Cards” (Qiagen, Hilden, Germany). Water Soluble Tape was placed in a beaker of water heated to 60 °C and agitated for 1 min. Once the DNA was eluted from the collection device, the standard DNA extraction procedure using the QIAGEN® QIAamp DNA Kit was used to extract DNA from all samples collected.[17] The extraction procedure was standardized to minimize any variability that may be introduced due to differences in the extraction procedure. During the last step of each extraction process 50 μl of nuclease-free water was used to elute DNA.

Real-Time qPCR analysis using the Promega® PlexorHY Human Quantitation kit (Promega, Madison, WI) was performed on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) instrument following the manufacturer's procedure to determine the amount of DNA present in each sample.[18] Each sample was quantified two times to increase the accuracy of the estimated DNA amount; if a CT difference greater than 0.5 was observed between the replicates then the quantitative analysis was repeated.

Thirty-two samples were selected from both studies for Short Tandem Repeats (STR) analysis; eight pairs were selected from the double swabbing vs. tape lifting study and eight pairs were selected from the double swabbing vs. FTA paper scraping study. Samples were selected with DNA amounts of at least 1 ng total DNA. Only three DNA samples contained less than 100 pg/μl; these samples were concentrated using heat to allow 1 ng DNA input. STR analysis using the AmpFLSTR Identifiler™ kit (Applied Biosystems, Foster City, CA) was performed to generate a driver's profile from the 32 selected steering wheel samples and the 16 corresponding buccal swabs. The manufacturer's recommended procedures were followed for amplification using a Gene Amp® PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA) at the recommended 28 cycles and analyzed using a ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). GeneMapper®ID software (Applied Biosystems, Foster City, CA) was used for STR data analysis. The analytical threshold was set to the recommended 50 RFUs and the stochastic threshold was set to 150 RFUs.[19] Results from the STR analysis were analyzed for mixed profiles and used to assess the completeness of STR profiles. DNA collection methods were compared based on how well they recovered the STR profile of the most recent driver by counting the number of observed alleles. The goal of the STR analysis was to assess the difference between quantifiable and typable results among the three collection methods.

2.3. Statistical analysis of data

The data were $\log_{10}$ transformed in order to improve the normality of variables. A Wilcoxon Signed Rank test was performed in order to assess the significance levels between the DNA yields