

Sodium Chloride as a Swabbing Solution for DNA Collection from Latent Fingerprints

Ludmila Alem*

Department of Forensic Science and Technology, State University of Rio de Janeiro, Rio de Janeiro, Brazil

Abstract

Latent fingerprints are a common investigative tool used not only by police forces, but also by military forensic experts. One of the most routinely forensic investigation conducted within the Brazilian army is due crimes against the patrimony where fingerprint analysis is an efficient way of identifying perpetrators. DNA analysis of smudged or incomplete prints can be a complementary methodology making full use of the evidence. Considering the context of crime scene analysis within the Brazilian army, we assessed the use of sodium chloride 0.9% (NaCl) as a swabbing solution for DNA collection from fingerprints deposited on glass and metal surfaces combined with lysis solution methodology for DNA extraction. Also, we compared the results obtained from using Sodium Dodecyl Sulfate 2% (SDS), a common choice of swabbing solution. The data found in this study showed no statistically significant difference regarding the recovery of DNA from latent fingerprints between the two tested solutions. However, the use of NaCl 0.9% as a collection solution combined with lysis solution as an extraction method presents an advantage of less time-consuming and lower costs overall.

Keywords: Crime scene • Military • Latent fingerprints • Forensic biology • Forensic DNA typing • Swabbing solution • Touch DNA

Introduction

Fingerprint analysis is one of the oldest human identification techniques used in forensic caseworks. Fingerprint features are a valuable evidence that can link an individual to a crime through dactyloscopy analysis and serve as a source for DNA that was transferred by contact. In a non-controlled environment, such as a crime scene, latent fingerprint may be smudged resulting in the loss of friction ridge details leading to insufficient data that are not suitable for comparison with a questioned sample [1]. In this context, an attempt to generate a genetic profile from the fingerprint it is a valuable approach widely investigated to make full use of the evidence [2].

Improvements on DNA recovery and genetic analysis methodologies for latent fingerprints may be achieved with better understanding regarding its origins and nature. The DNA transferring mechanisms from the skin surface still remain a subject of study. Some authors claim that skin cells are nucleated and naturally release DNA during the desquamation process while others postulate that during the skin cellular differentiation (keratinization) cells undergo gradual nucleus shrinkage and chromatin condensation becoming DNA free

as they migrate through skin layers and therefore, they could not donate DNA to the surfaces. It has also been postulated that nucleated skin cells in latent fingerprints are transferred from other body areas. Also, some studies advocate that there is a residual quantity of DNA in keratinocytes due to incomplete DNA degradation during the differentiation process.

Nevertheless, the deposition of DNA on objects at a crime scene is intimately related with the skin proliferation rate, which varies between individuals. Based on their propensity to shed skin cells through contact, individuals can be characterized as good and bad shedders, with the first ones often releasing enough DNA to generate a full genetic profile. Bad shedders will likely return a partial or negative genetic profile [3]. Environmental conditions (humidity, temperature, light exposure) and the type of surface on which DNA is deposited (porous and non-porous substrates) will influence the DNA recovery: Porous substrates as wood and fabric are more absorptive than metal and glass (non-porous substrates).

One of the most common types of crimes processed by military forensic experts are the ones against the patrimony including burglary of cabinets in military barracks, safes, armaments and ammunition. In these types of crimes, the surfaces

*Address for Correspondence: Ludmila Alem, Department of Forensic Science and Technology, State University of Rio de Janeiro, Rio de Janeiro, Brazil; E-mail: ludmila.alem@msn.com

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surfaces are searched for fingerprints or collected for touch DNA analysis. If this is the case, the military forensic experts are advised to use the double swab technique for evidence collection. However, crime scene analysis in the scope of the Brazilian army are conducted in a vast variety of environments alongside the Brazilian territory. Most of the places where the crime scene analysis is developed commonly lack the minimal resources for adequate collection and preservation of biological samples [4]. Yet, the samples are exposed to high humidity and high temperature and the time interval between the sample collection and its arrival to the DNA laboratory is often inadequate due the distances between these places.

In order to facilitate the collection of biological samples when double swab technique is needed, we analyzed the performance of sodium chloride 0.9% as a collection solution. The NaCl 0.9% solution is easily purchased from pharmacies and supermarkets, easily transported and stored and is a low-cost solution. We evaluate its performance in combination with lysis solution extraction methodology, a less time-consuming and low-cost methodology. Experiments were also conducted with SDS 2%, a regular swabbing solution.

Materials and Methods

Preparation of reference samples

For the purpose of this study, blood samples of 20 volunteers were collected on FTA[®] classic cards and used as a reference sample for the comparison with the genetic profiles obtained from latent fingerprints deposited on glass and metal surfaces [5]. A punch of 1.2 mm of diameter was selected from the cards and submitted to the following wash protocol: 198 μ l washing buffer (10 mM Tris 0.1 mM EDTA 0.5% SDS), 2 μ l proteinase K 20 mg/mL. Samples were left overnight at 37°C. The solution was discarded the following day and the punch left to incubate with 200 μ l of washing buffer for 5 minutes at room temperature (repeated at least 3 times). Solution was discarded and replaced by 200 μ l of milli-Q[®] water (incubation at room temperature for 5 minutes, repeated at least 3 times). All water was removed from the tubes and punches were left to dry at 55°C for 30 minutes with open tubes. STR amplification was performed with AmpFISTR[®] Identifiler[™] PCR Amplification kit (life technologies) according to the manufacturer's recommendations.

Solution and substrate preparation

Sodium chloride (NaCl) 0.9% and Sodium Dodecyl Sulfate (SDS) 2% used as swabbing solutions were prepared according to manufacturer's instructions and sterilized in an automatic autoclave stermax Top[®] (121°C, 30 minutes).

Microscopy glass slides and microscopy glass slides wrapped in aluminum paper to mimic metal surfaces were selected as substrate types [6]. They were cleaned with sodium hypochloride 5%, followed by ethanol 70% and UV sterilization for 20 minutes prior to fingerprints deposition.

Fingerprint transfer and recovery

Volunteers pressed their index finger on each surface for 30 seconds. Before donation of fingerprints, informed consent was obtained from all individual participants included in the study. To proceed with fingerprint collection, volunteers were asked to rub the index fingers through their faces and hair to obtain a higher DNA yield with more cells to be transferred. A total amount of 160 fingerprints were processed for this study: 40 fingerprints for each combination of swabbing solution and substrate type. Sterile swabs were moistened with 120 μ l of NaCl 0.9% or SDS 2%. To collect the fingerprints deposited in each substrate (glass and metal), rotating movements were used, allowing the entire cotton swab area to be in contact with the fingerprint. A dry swab was applied to each surface after the first swab. This double swab technique was used to maximize cell collection and DNA yield [7].

DNA extraction from fingerprints

Cotton from both swabs (moistened and dry) was cut with a scalpel blade, added to individual microcentrifuges tubes and submitted to DNA isolation with lysis solution (192.8 μ l of SDS 0.05%+7.2 μ l of proteinase K 20 mg/mL) according to the following protocol: 56 °C for 30 min at 1400 RPM, 100°C for 10 min (no agitation) and 4°C for 5 min. The concentration of the extract- the liquid volume obtained at the end of the DNA extraction protocol was approximately 200 μ l for each swab and the removal of the SDS was accomplished through 10 minutes of centrifugation in microcon[®] centrifugal filters 0.5 mL 100 K (merck millipore) pretreated with 1.0 ng of poly (A) RNA according to the manufacturer's instruction, generating 19 μ l as a final volume [8]. During this step, the DNA extract from both swabs was concentrated in the same microcentrifuge tube: Through each microcon[®] centrifugal filter, approximately 400 μ l of DNA extract was filtered.

Quantification and STR analysis

Quantification was carried out using the quantifiler duo DNA quantification kit and steponeplus[™] real-time PCR system (applied biosystems). DNA extracts were amplified using the ampflstr[®] minifiler[™] PCR amplification kit (life technologies) and a veriti[®] 96-well thermal cycler according to the manufacturer's instructions.

PCR products were submitted to capillary electrophoresis on the ABI prism[®] 3500 genetic analyzer and the data was analyzed considering a threshold of 175 RFU in the genemapper[®] ID software v1.2 (applied biosystems).

Results

The results obtained from the quantification assay indicate the SDS 2% as a better collection solution when compared with the results from the NaCl 0.9% solution for both surfaces examined. The DNA mean concentrations were found to be higher in fingerprints deposited on glass despite the collection solution tested [9]. Also, high standard deviation values were obtained for these analyses (Table 1).

Swabbing solution	Surface	DNA concentration range (pg/μL)	DNA mean concentration (pg/μL)	Standard deviation
NaCl 0.9%	Metal	8.5-152.3	38.24	33.3
SDS	Metal	2.8-700	78.11	151.2
NaCl 0.9%	Glass	10.0-140.0	48.4	36
SDS	Glass	8.8-980	93.34	194.7

Table 1. Quantification data assay obtained from fingerprints deposited in glass and metal surfaces and collected with different swabbing solutions. The number of samples equals 40 for each combination of swabbing solution and surface. NaCl 0.9%=sodium chloride, SDS 2%=Sodium Dodecyl Sulfate.

Regarding these observations, we decided to evaluate the overall success of DNA recovery in each group combination based on STR analysis. Considering sixteen alleles the total number of alleles possible to be recovered for each volunteer within each assessed group, the results obtained are shown in Table 2.

Swabbing solution	Surface	Allele rate recovery
NaCl 0.9%	Metal	0.9859
NaCl 0.9%	Glass	0.9969
SDS	Metal	0.9969
SDS	Glass	0.9984

Table 2. Allele recovery rate obtained from fingerprints deposited in glass and metal surfaces, collected with different swabbing solutions. The number of samples equals 40 for each combination of swabbing solution and surface. NaCl 0.9%=sodium chloride, SDS 2%=Sodium Dodecyl Sulfate.

The statistical analysis applied to these data returned a p value greater than 0.05 (p value=0.439). The post-hoc test of steel-dwass method was also applied and the results indicated no statistical differences between the swabbing solutions tested regarding the genotyping analysis. Also, no statistical differences were observed regarding the type of surface evaluated. All p values were greater than 0.05 (Table 3).

Groups analyzed	Variable analyzed	p value
Metal+NaCl 0.9% vs. Metal+SDS 2%	Swabbing solution	0.278
Glass+NaCl 0.9% vs. Glass+SDS 2%	Swabbing solution	0.937
Metal+ NaCl 0.9% vs. Glass+NaCl 0.9%	Surface	0.278
Metal+SDS 2% vs. Glass+SDS 2%	Surface	0.937

Table 3. Comparison of 2 to 2 (pairwise) test utilizing steel-dwass data for the number of alleles recovered.

Electropherogram for the samples shows absence of contamination, well defined and balanced peaks in concordance with quality standard values determined by ABI 3500 platform (peak height above 175 RFU and results within the range quality 0.75 and 1.00) for the ampflstr® minifiler™ PCR amplification kit (life technologies) loci recovered. Electropherogram obtained for one fingerprint deposited on the metal surface and collected with NaCl 0.9% as a swabbing solution is showed as an example (Figure 1).

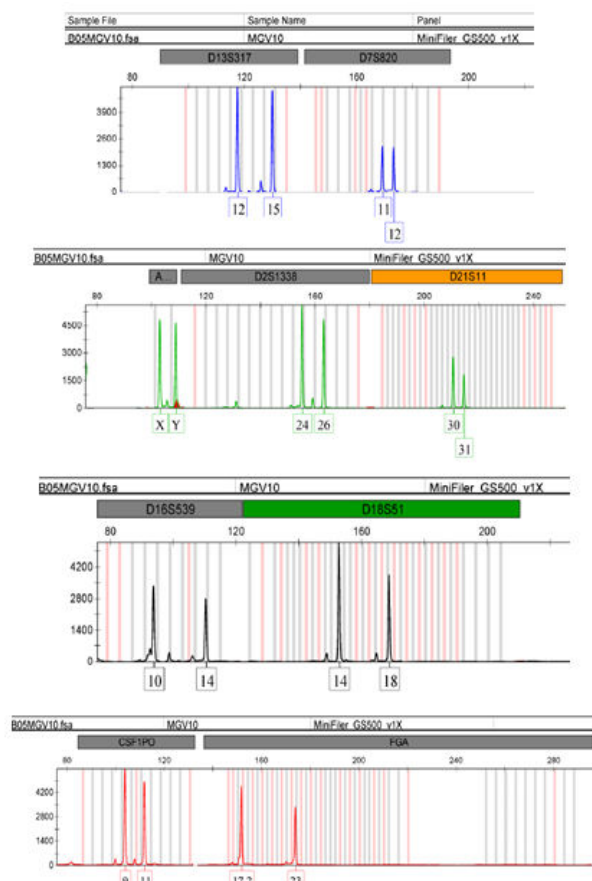


Figure 1. Representative electropherogram obtained from a fingerprint deposited on the metal surface and collected with NaCl 0.9% as a swabbing solution. DNA extracts were amplified with ampflstr® minifiler™ PCR amplification kit (life technologies).

Discussion

The genetic analysis of fingerprints beyond dactyloscopy parameters as a touch evidence is notably important in forensic investigations. In the absence of expected biological fluids such as blood, saliva, semen, hair or urine, touch DNA evidence will still be left behind after the touching due to the natural process of skin desquamation. Therefore, when considering touched surfaces (cellphones, firearms, car steering wheels, kitchenware etc.) as admissible casework samples for genetic analyzes, the sample space is expanded and the search for the perpetrator's identification may be successful. Raymond et al., compiled 252 samples collected from touched surfaces in 201 robbery cases, assaults and drug felonies during one-month period which corroborates the idea of expanded sample space. Also, most traces found at crime scenes are contact related and outnumber biological fluids.

As is demonstrated by the high standard deviations for the DNA mean concentrations obtained from our samples, the amount of DNA transferred to the surface from fingerprints varies among individuals as it is expected. Farmen et al., affirms that people may be good or bad DNA donors according to their capability of transferring epithelial cells in the

sweat. When a crime scene is being processed the potential sources of touched evidence are usually dry. The use of wet swabs in these cases allows cells to be rehydrated and detached from the surfaces more easily. Different types of swabbing solutions are widely used. In the U.S. for example, water is commonly used for this purpose. Triton X-100, SDS and tween 20 are also options of solutions. Thomasma and Foran show that when a substance with detergent properties is present in the swabbing solution as SDS, the DNA yields from fingerprints are higher. However, if the samples are not readily processed, the chemical properties of SDS can induce DNA degradation and the lack of purification may inhibit the polymerase chain reaction. On the other hand, NaCl 0.9% is an aqueous solution that maintains cells integrity and it resembles physiological conditions sustained *in vivo*, possibly contributing to avoid that cells degrade so quickly.

In the military scenario, the majority of forensic experts do not have molecular biology training or easy access to laboratories. The only laboratory available to fulfill forensic DNA testing requests for the Brazilian armed forces is the army's biology institute-IBEx, located in the Rio de Janeiro city. Therefore, it is not unusual to have extended wait times between evidence collection and genetic analyzes. Taking this into account, the use of a solution that does not contain any potentially DNA degrading compounds such as detergents could be a better option for this routine. Also, NaCl 0.9% is widely available commercially and easily purchasable as the cost of using it is 10 times lower when compared with SDS 2%.

DNA yields and allele detection were slightly smaller for samples collected with sodium chloride but we found no statistical differences between SDS 2% and NaCl 0.9% as swabbing solutions. Therefore, they were both efficient as swabbing solution for DNA collection. An adoption of NaCl 0.9% does not impair the possibility of successful results and should not be disregarded [10].

Concomitantly, we believe that when dealing with touch DNA evidence, a DNA extraction methodology that involves less sample handling would provide improved results regarding successful DNA typing. As a comparison, column DNA extraction kits requires several steps including tubes changing, buffer washings, column centrifugation steps, which could result in small DNA losses as we have observed from previous works (data not shown).

On the other hand, the lysis extraction methodology is basically one step process: After cutting the entire cotton tip of the swab into a microcentrifuge tube and the addition of the DNA extraction solution, the sample is not handled until the end of the protocol [11]. Then the extracted DNA can be transferred to a new microcentrifuge tube and it is ready for downstream applications. The processing of samples with microcon® centrifugal filter devices proved to increase the number of loci obtained by allowing more DNA to be added to the final reaction and this is associated with the increase of DNA: PCR inhibitors ratio. Nevertheless, the DNA extract obtained after completing this protocol was concentrated with Microcon® 100K centrifugal

filter units 100K, increasing the probability of generating a full profile. Also, the addition of 1 ng of poly (A) RNA to the microcon® membrane improves the DNA recovery during concentration step as it prevents DNA loss. Besides fewer sample handling steps, the costs for the lysis extraction protocol is considerably lower than commercially available kits, is less time consuming and less laborious. And as observed in the results, this type of DNA extraction methodology is suitable for touch DNA evidence [12].

As observed in the quantitative assay, the amount of DNA found on the fingerprints is in the order of picograms. Other evidences, such as blood, semen or saliva usually return nanograms of DNA. In cases where the target DNA is frequently so low, workflow optimization plays an important role. Therefore, more valuable than DNA quantity inherent to each type of evidence, it is the concern to prevent unnecessary DNA losses, choosing the most suitable extraction methodology, ideal storage and caution during evidence collection. The use of NaCl 0.9% as a swabbing solution in combination with lysis extraction methodology generated good results and represents a routine workflow optimization regarding DNA analysis within the Brazilian armed forces.

In addition, according to Daly et al., the type of surface is a strong variable that affects nucleic acids recovery. In our study both substrates (glass and metal) were non-porous surfaces, which may be one of the reasons why the genotyping results did not show statistical differences concerning the success or quality of the analysis. Satisfactory STR profiles were obtained for both surfaces under our tested conditions.

Conclusion

Our results showed that no statistical differences were found between the swabbing solutions studied. However, we recommend the use of sodium chloride 0.9% solution in combination with lysis extraction methodology for processing touched DNA evidence whenever low-cost and less-time consuming methodology are necessary. It was possible to obtain complete profiles from the majority of latent prints analyzed. We observed no statistical differences between glass and metal surfaces regarding all groups tested. However, it must be considered that these results were achieved under a controlled environment and samples collected from crime scenes may produce different results as they are under the influence of several not controlled factors. The choice of sodium chloride 0.9% as a swabbing solution followed by a lysis extraction methodology represents a good workflow for genetic analyzes of

fingerprints in forensic casework within the Brazilian army. Since the development of this study, several cases were successfully processed utilizing the suggestions to touch DNA evidence protocols presented here.

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