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A STUDY ON EFFECTS OF IMMERSION IN RIVER WATER AND SEA WATER ON DNA PLACED ON GUNS

Original scientific paper

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Abstract

To determine the quantity of DNA that can be obtained from that placed on guns after 30 minutes, 90 minutes and 270 minutes of immersion in water. Also, the aim of this study was to disclose how different types of water environments, such as freshwater and saltwater, affect the amount of DNA loss and degradation over the preset period of time.

Firearms was swabbed with a double swabbing technique. DNA was isolated using a Qiagen procedure, quantified by real-time PCR, amplification was on thermal cycler and Capillary electrophoresis was performed on 3500 Genetic analyzer. The electropherograms obtained were analyzed with the GeneMapper ID-X software.

Through the comparison of DNA amounts detected after isolation and DNA profiles/allele numbers detected on guns, it can be concluded that the influence of saltwater and freshwater are significantly different. The loss of DNA in saltwater from the outer firearms surface after 30 min is similar to the loss of DNA in freshwater after 90 minutes of immersion.

Based on the results of this study, especially the results of real casework sample, it can be concluded as expected that saltwater has a more serious effects on the degradation and the loss DNA on guns than freshwater. Further, it point out that the guns found in freshwater after crime could be a valuable piece of forensic evidence, whilst the guns recovered from saltwater will have less value as forensic evidence if found after more than 30 minutes

Keywords

forensic analysis, trace DNA, firearms, effects of aqueous environment

1. INTRODUCTION

Trace DNA analysis is an important tool in crime scene investigation. Possibility to detect the biological materials is very significant, but it depends on quantity of DNA extracted. It is understandable that touch DNA samples typically contain low amounts of DNA and are prone

to degradation and loss due to different environmental condition, but importance of such samples emerges since nowadays more than 50% of samples are of that kind.

Every contact with persons and items leaves some biological material. "Touch DNA" usually can be transferred via skin. It is assumed that touch DNA originates from several sources. Small amounts of DNA that are present on the surface of the skin originates from keratinocytes sloughed from upper epidermal layers (Marieb, 2011). The presence of sweat also contributes to touch DNA sample; along with the cell-free nucleic acid which also contributes to a total amounts of DNA, as well as corneocytes and other types of nucleated cells (Kita, Yamaguchi, Yokoyama & Tanaka, 2008).

Factors which affected amounts DNA for transfer				
Shedder status	Some individuals leave higher amount of biological material than other. The are "good shedders" (Phipps i Petricevic, 2006).			
Hand washing	Hand washing would remove biological materials (Goray, Mitchell & Van Oorschort, 2010).			
Personal habits	Frequent touching of face, eyes, nose, causes transferring DNA from those areas to the hands (Ryan, 2016).			
Type of contact	Pressure and friction affects the amount of transferred DNA (Goray et al, 2010).			
Substrate	Rough surface retains more skin cells than a smooth surface (Wickenheiser, 2002)			
Perspiration	Sweat could increase amount of DNA. When sweat passes through pores it could collect cells along the way. Also sweat contains cell–free nucleic acid and epithelial cells (Ryan, 2016).			

Table. 1 – Factors which affected amounts DNA for transfer.

Biological traces are deposited differently on different surfaces and also persist in a different way due to environmental conditions. Rough objects that have cracks, crevices or grooves might better collect skin cells and thus protect DNA in time (Wickenheiser, 2002). After placement of objects indoors, it is expected that the DNA stay longer than if placed outdoors, since it is exposed to heat, humidity, water, UV light, bacterial growth, oxidizing agents (Raymond et al, 2009). DNA degradation on biological material could be affected by chemicals (oxidizing agents, acid, base), physical (heat, humidity) and biological agents (bacterial growth) (Lowe et al, 2002). Analysis of DNA on firearms is very important due to outgrow of offences that involve use of it and further consequences that such use produce. Potential of DNA to produce valuable information in the investigation has been demonstrated on guns' grip panels, slide and magazine of the gun (Polley et al. 2006). During such analysis, a forensic laboratory often has to deal with samples that contain low quantity of DNA, since the evidence may have been exposed to hard environmental conditions that may speed up the degradation of DNA.

Among other ways, experience shows that firearms (although it does not happen frequently), can be, after the offense, disposed of in an aqueous environment which can influence degradation and loss of DNA placed and thus to pose a problem for future investigations.

The purpose of this research was to determine the quantity of DNA that can be obtained from DNA placed on guns after 270 minutes of immersion. Also the aim of this study is to determine how different types of water environments such as freshwater and saltwater affect the amount of DNA loss and degradation over the preset period time, and to show how rapid the concentration of DNA decreases depending on initial concentration and environmental condition.

2. MATERIALS AND METHODS

2.1.1 Experiment

Five people (three female and two male) volunteered for two series of experiments. Their reference samples were collected on FTA cards and DNA profiles obtained for future comparison.

Six guns were obtained for this study. The guns were wiped with 1% sodium hypochlorite solution and afterwards with 70% ethanol before deposition of DNA. Then, participants were asked to carry guns for two days, trying to leave as much DNA as possible. This method for DNA depositions was used in every series of the experiment

	Characteristic of guns
Gun1-T1/1	"Reck Miami 92 F "black cal.9mm, gun's grip panel plastic, full metal body, without fabric number
Gun2-T1/2	Same characteristic like gun No.1
Gun 3-T2	"Norica Compact M 2002", black, cal 9 mm with fabric number 7-54240, with gun's grip panel, blown.
Gun 4-T3	"Herstal Belgique", brown, fabric number 29881, wooden grip, full metal body.
Gun 5-T4	Marked "Mauser Werke A.Q. Oberndorf, cal 6.35 mm, fabric number 27783, magazine with perforation, full metal
Gun 6-T5	Marked "P.Berretta 1936 with Serial Number 516580. There are a few markings under the grips, what appear to be a "PB", metal body, gun's grip from one side wooden.

Table 2 – characteristic	of	guns
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The primary goal of this study was to analyze how the concentration of DNA on firearms decreases with increasing time of deposition in water environment. Two guns were of the same type (marked with same first number), and handled by the same person, and were used to compare the influence of freshwater and saltwater. Other guns had different characteristics, and were handled by different persons.

In series A three sets of experiments are designed: five of the guns were exposed to the effects of freshwater from the river for three different time periods (30 minutes, 90 minutes and 270 minutes). The remaining gun was exposed to the saltwater for same three different time peri-

ods (30 minutes, 90 minutes and 270 minutes).

The amount of DNA and DNA profiles detected on guns before exposure to water conditions, were used as reference.

Each participants had one gun, only one of them had two guns, same type and model (Table 3).

	Person 1	Person 2	Person 3	Person 4	Person 5
Gun 1-T1/1	х				
Gun 2-T1/2	х				
Gun 3-T2		х			
Gun 4-T3			x		
Gun 5-T4				х	
Gun 6-T5					х

Table 3 – illustrate use guns

After deposition, samples, overall 48, were collected with double swab technique from two position: external position - from gun's grip panels and from the slide internal position - from the entire surface the magazine of the guns.

In the series B three set of the experiments were designed using same guns from series A, now immersed in saltwater from the sea, to investigate effects of saltwater on the DNA. The same submersion time periods were used as in series A. The gun marked as T1_2 was not used in this series.

Samples, overall 30, were collected with the double swab technique from the same position as in first series

2.1.2 Casework sample - The gun from the stream

Felony of unlawful possession of firearms - the gun (black "Pietro Beretta" cal. 7.65 mm) was disposed of in brackish water (a mixture of salt and fresh water). It was found in, by a police officer, 120 minutes after the suspect disposed of it and was collected as an evidence. Two positions on the external surface were sampled with the swab technique: - gun's ribbed grip panels and from the slide of the gun; and from internal surface of magazine. Afterwards, one sample was composed out of these two swabs.

Suspect DNA was collected on a FTA card and DNA profile was obtained for future comparison.

2.1.3 Quality control

The guns were decontaminated with bleach to avoid contamination.

All buffers and plastic used was UV crosslinked, and negative controls were used to detect potential laboratory contamination. Both positive and negative controls were implemented for every reaction during DNA processing steps.

2.2 Methods

FTA purification was using the standard FTA card purification protocol employed by the BCIT Forensic (DNA extaction from FTA cards, 2022).

Double swabbing technique involved a moist swab (with few drops of ultra pure water), followed with dry one (Sweet et al, 1997).

DNA extraction was performed using QIA AMP INVESTIGATOR KIT (QIAamp DNA Investigator Handbook, 2020). All samples were incubated for a total of 24 h at 56° C. Finally, DNA was eluted in a elution buffer in final volume of 30 μ l.

Quantification was performed with Quantifiler Human Kit on Real Time PCR instrument. Quantification reaction mix was prepared according to manufacturer's recommended protocol (QIAamp DIH, 2020).

PCR amplification was performed with AmpFI STR NGM kit on Thermal cycler 9700 Applied Biosystem following the manufacturer's recommended protocol (AmpFISTRNGM PCR Amplification Kit, User Guide, 2012), in final volume of 25 μ l.

Even samples which showed undetected result in quantification were amplified, using a maximum volume of 10 μ l (Absolute Quantitation Using standard Curve, 2006).

Capillary electrophoresis was performed on 3500 Genetic analyzer (3500350xl Genetic analyzer with series Data Collection Software 3.1 User Guide, 2015). Analysis method was used, whose parameters are specified by the manufacturer and were internally validated in the lab. The samples were separated on a 36 cm capillary array. The amplified products were electrokinetically injected on 3500, 8 capillary Genetic Analyzer using POP 4. Data was analyzed using a peak detection threshold of 110 rfu for green and blue base line, 175 rfu for yellow and 200 rfu for red base line. The electropherograms obtained were analyzed with the GeneMapper ID-X software. All methods were internally validated in DNA lab.

3. RESULTS

3.1 Recovery of DNA amount and profile analysis from guns used as reference

The mean DNA yield recovered from guns was 7.791 ng/ μ l (range 0.03 to 42 ng/ μ l). The mean DNA yield collected from external surfaces (gun's grip panels and slide) was 14.1075 ng/ μ l (ranged 0.215 to 42 ng/ μ l), while the mean DNA yield from internal surfaces (entire surface of the magazine) was 0.21248 ng/ μ l (ranged 0.03 to 0.49 ng/ μ l).

For eleven samples, complete profiles, from external and internal position on guns matched with the DNA of the donors.

Series A

3.2 Recovery of DNA amount and profile analysis from guns submerged in fresh water for 30 minutes

The mean DNA yield collected external surfaces of guns (gun's grip panels and slide) was 0.7922 ng/ μ l (ranged from 0.181 to 1.86 ng/ μ l). The mean DNA yield from internal surfaces (entire surface of the magazine) was 0.106 ng/ μ l (ranged from 0.035 to 0.213 ng/ μ l). Ten samples gave complete profiles, and matched with the profiles of donors.

3.2 Recovery of DNA amount and profile analysis from guns submerged in fresh water for 90 minutes

The mean DNA yield collected from the external surfaces was 0.4372 ng/ μ l (ranged from 0.075 to 0.541 ng/ μ l), while the mean DNA yield from internal surfaces 0.072 ng/ μ l (ranged from 0.0192 to 0.118 ng/ μ l).

All ten samples gave complete profiles, and matched with the profiles of donors.

3.2 Recovery of DNA amount and profile analysis from guns submerged in fresh water for 270 minutes

The mean DNA yield collected from the external surfaces was 0.3058 ng/ μ l (ranged from 0.0776 to 0.933 ng/ μ l). The mean DNA yield of internal surfaces was 0.1104 ng/ μ l (ranged from 0.00138 to 0.406 ng/ μ l).

All samples gave complete profiles, and matched with the profiles of donors.

Series B

3.2 Recovery of DNA amount and profile analysis from guns submerged in salty water for 30 minutes

The mean DNA yield collected from external surfaces of guns submerged in salty water for 30 minutes was 0.3931 ng/ μ l (ranged from 0.075 to 1.05 ng/ μ l). The quantity of DNA on internal surfaces was mostly "not detected", with one sample that gave 0.00516 ng/ μ l.

Four complete profiles, one partial profile with 6, and one partial profile with 9 loci was detected, all that matched with the DNA of the donor.

3.2 Recovery of DNA amount and profile analysis from guns submerged in salty water for 90 minutes

The mean DNA yield collected from the external surfaces of 0,1625 ng/ μ l (ranged from not detected to 0.424 ng/ μ l). The concentration of internal position from the entire surface of the magazine of the guns was undetected.

Four complete profiles was obtained from external matched the DNA of the donor, and one sample gave partial profile, with result on only 1 locus.

No DNA profile were obtained from samples collected from internal surfaces.

3.2 Recovery of DNA amount and profile analysis from guns submerged in saltwater for 270 minutes

The mean DNA yield collected from the external surfaces from guns submerged for 270 minutes in salty water was of 0.07 ng/µl (ranged from 0 to 0.146 ng/µl). The concentration in samples from internal surfaces undetected.

Only one complete DNA profile was obtained from DNA collected from external surfaces of the guns that was in salty water for 270 minutes. Whole profile matched to the donor. Also, two partial profile with 11 and two with 5 loci recovered and matched with the DNA of the donors from same positions.

No DNA profile were recovered from internal surfaces.

3.3. Casework samples

The detected concentration was a 0.0461 ng/ μ l of DNA on samples from guns. The interpretable DNA result that was obtained from the sample on the gun is a mixed DNA profile from a minimum of three persons as donors. The reference profile from the suspect was completely included in the mixed DNA profile.

4. DISCUSSION

Biological evidence could be collected from the guns which are often used for DNA analysis.

This study, along with that of Polley et al. (2006) and a study conducted by Richert (2011). demonstrates that the grip of the gun and the slide could be optimal areas for obtaining useful profiles. In the study conducted by Nicholas Richert (2011) for the combined swabbing group DNA, which is consisted of samples created by combining the swabbing from individual swabbed regions on firearms, yield value of 4.4 ng (range 0.3–20.6 ng) was reported; and for the individual swabbing group DNA yield value of 1.8 ng (range <0.02–14.4 ng) was observed. Similar individual swabbing DNA yield values were reported in a study conducted by Polley et al. (2006). The mean DNA yield from the exterior location from firearms averaged 1.1 ng per sample with a range of 0.24 to 7.2 ng.

In this study the amount of DNA value from the external and internal positions was significantly greater. This could be attributed to the fact that grip, slide and magazine of the gun are the areas that an individual would hold and touch most frequently and for the longer periods of time. Also, participants were asked to hold firearms longer than in other studies, in order to obtain a higher initial DNA concentration. After cleaning, the guns were held by a single person. They were sampled immediately after a deposite of biological material, submerged in water and airdried shortly after. In a study conducted by Polley (2006), two persons loaded the same firearm and discharged five rounds so they placed less amount of DNA. Differences between amount of DNA from the external and internal positions were observed. The higher amount was obtained from external position.

As expected, we observe decrease in DNA present on the surfaces after water submerging. After fresh water submerging, the mean DNA yield collected from the external surfaces after 30 minutes, in (0.7922 ng/ μ l) that represented more than 17 fold decrease in comparison to the

starting average DNA quantity of (14.1075 ng/ μ l). After 90 minutes was 0.4372 ng/ μ l, which means more than 32 fold decrease, and in comparison to the starting average quantity of DNA from external position (at time zero), and after 270 minutes, was 0.3058 ng/ μ l, thus more than 46 fold decrease in comparison to the starting average quantity of DNA.

From internal surfaces, the mean DNA yield was 0.106 ng/µl, thus showed 2 fold decrease in comparison to the starting average quantity of DNA (0.21248 ng/µl)) after 30 minutes, after 90 minutes was 0.072 ng/µl, which is approximately 3 fold decrease, and after 270 minutes heures was 0.11 ng/µl, again approximately 2 fold decrease in comparison to the starting average quantity of DNA.

The period of time spent in fresh water had a significant influence, greater for the external surfaces of guns. With longer time interval loss of target DNA on external position on guns was more prominent. On the other side, significant decline of the amounts of DNA from internal position of guns was not observed.

The mean DNA yield collected from the external surfaces after the effect of saltwater for 30 minutes was $0.3931ng/\mu$ l, which means more than a 35 fold decrease (at time zero was 14.1075ng/µl). Further, the mean DNA yield after the effect of saltwater for 90 minutes, was of 0.1625 ng/µl (a 86 fold decrease) and after 270 minutes, was of 0.07 ng/µl, (more than a 201 fold decrease over the starting average quantity of DNA). The DNA quantity of samples from the internal position from guns was mostly "not detected", and only one sample was 0.00516 ng/µl after 30 minutes of time, although the starting average quantity of DNA from internal position (at time zero) was 0.199 ng/µl. The time period of submersion of guns in saltwater had a significant influence. Only 30 minutes of submerging gave adequate quantities of DNA amounts, collected from external surfaces, for further analysis. Surprisingly, the amounts of DNA extracted from the internal surfaces position was very low, showing substantial loss of biological material.

With comparing of DNA amounts and profiles obtained, in case of saltwater and freshwater, we got significant difference. For example, the loss of DNA in saltwater from the external surfaces after 30 min is close to the loss of DNA in freshwater after 90 minutes of submerging. Further, samples from internal surfaces of guns immersed in saltwater showed such extensive DNA degradation and loss that it was even unable to detected DNA.

In this study, in concordance with that of Graham (2014) we showed that the saltwater submerging influenced more DNA loss. Increased salinity inhibits the effects of hydrolysis, but increases cell lysis, in which process DNA could be released at the end freely in the environment. It should be noted that in the study conducted by Emma Graham (2014), there was a significant DNA degradation and loss in both tissue and bone samples which were immersed in saltwater and freshwater but for longer period of 72 h.

In the study by Claire Gillespie (2018), it was shown that rusting of the metal is all about the movement of electrons and that iron rusts more quickly in salt water than it does in freshwater. So, higher amounts of corrosion were detected in saltwater comparing to freshwater in shorter submersion time. This can be explained with that saltwater, an electrolyte solution, contains more dissolved ions than fresh water, meaning that electrons can move more easily. Corrosion on metal further affects degradation of DNA on guns. Similarly, extremely corrosive chemicals can break the hydrogen bonds between DNA base pairs and thus degrade or "denature" a

DNA sample. In general, the seriousness of degradation mainly depends on the material of the gun, submersion time, condition of the environment and time of corrosion, along with starting quantity of DNA. In other words, the quality of genotyping depends largely on the degradation processes of the DNA molecule that has been exposed to. Degradation accumulates with time while environmental conditions (temperature, humidity, pH, soil chemistry) can modify the rate and aggressiveness of degradation (Fondavila et al, 2008). Factors such as the development of inhibitors (e. g. rust on metal) and prolonged exposure to environmental insults can produce differences between rate of degradation (Bushra, 2016).

The casework presented inspired the development the experiments. It indicated that the firearms which was disposed of in aqueous environment could yield meaningful results. Detected concentration of DNA (0.0461 ng/µl) in this case was close to the mean DNA yield collected from the external surfaces of guns (0.07 ng/µl) after the submerging in saltwater for 270 minutes. As we saw in our case, the results of this study so demonstrated that profitable amount of DNA could be obtained from DNA samples collected from guns which were in water for some time and thus confirmed our actual findings in real casework.

5. CONCLUSION

The aim of study was to show how rapid the concentration of DNA was decreasing depending on initial concentration. The result of this study indicated that biological material could be successfully obtained from external and internal surfaces of guns submerged in water to generate partial or full DNA profile for forensic investigation. The guns were submerged in water in the laboratory environment, thus lacking real natural water stream.

Based on the results of this study, especially the results of real casework sample, it can be concluded as expected that saltwater has a more serious effects on the degradation and the loss DNA on guns than freshwater. Further, it point out that the guns found in freshwater after crime could be a valuable piece of forensic evidence, whilst the guns recovered from saltwater will have less value as forensic evidence if found after more than 30 minutes.

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