DNA Recovery and Transfer on Non-Porous Surfaces Submerged in Spring Water

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FBI ends search of lake in San Bernadino massacre investigation *(Luis Sinco / Los Angeles Times, 10 Dec 2015).*

1. Introduction

What Do We Know?

Submerged items are commonly thought to lack evidentiary value [1]. For instance, some investigators believe that all DNA could be lost once an item is exposed to a flowing current or tossed into a body of water. However, **previous studies have shown the ability to recover DNA from submerged porous items** for upwards of six weeks [2].

The crevices or interweaving fibers in porous items are thought to protect DNA from being washed away [2]. Smooth non-porous surfaces inherently lack the traits that might aid in DNA retention. Previous studies have shown that **alleles from stains on non-porous surfaces can still be detected up to three days submersion,** but allele dropout can occur as early as twelve hours into the submersion period [2].

What is the Problem?

As far as the authors are aware, studies have reported the percentage of alleles **but not the quantity of DNA recovered from submerged non-porous items.** After extracting a sample, a DNA analyst determines the quantity of DNA, which can correlate to the investigative viability of a sample after STR amplification. Some analysts may not wish to proceed with STR amplification if DNA quantities are too low, especially if the sample has been exposed to environmental conditions including submersion. **However, we wanted to see if samples from submerged non-porous items could hold more evidentiary value than many anticipate.**

2. Purpose

Hypotheses

Because non-porous surfaces do not have traits that might aid in DNA retention, then **DNA quantities and the number of alleles recovered will decrease over longer submersion periods.** 1

Because flow is capable of dislodging, then **DNA quantity and the number of alleles will decrease at a slower rate in stagnant water versus in a flowing current.** 2

Objectives

Determine and compare the **quantity** of samples submerged in stagnant water versus flowing water over one week.

Determine and compare the **alleles amplified** in samples submerged in stagnant water versus flowing water.

Observe possible **DNA transfer** from samples to blank surfaces submerged in water together over one week.

Observe a possible **correlation** between quantity of DNA and alleles amplified in samples submerged in water.

3. Methods

Sample Categorization

Samples were divided into two categories: **"deposition" and "blank" samples.** "Deposition" samples were glass slides that received a known amount of DNA. "Blank" samples were glass slides that received no donor DNA. Deposition samples were run in triplicate.

Sample Deposition

One donor was utilized for the study. Slides were cross-linked on each side to remove background DNA. A known amount of salivary DNA was deposited on each **deposition** sample glass slide i.e. 5uL of neat saliva of known quantity. Samples were submerged into stagnant or flowing spring water (flow rate approx. 10 cm/sec). **Deposition and blank samples were run alongside one another in the same vessel for the duration of the experiment.**

Sample Collection and Processing

Samples were **removed from their water conditions at t=6, 12, 24, 48, 72, and 168 hours**. Slides were air-dried and swabbed with a wet-dry swab method. Any DNA from the swabs was extracted (with QIAamp DNA Investigator Kit) and quantified (with Quantifiler Human Kit). STR amplification (with Globalfiler PCR Amplification Kit) was performed on one deposition sample and blank sample from each time period. Electropherograms were viewed and interpreted with GeneMarker HID STR Human Identity Software.

4. Results

DNA Quantity

Neat saliva was deposited onto slides, allowed to air-dry, swabbed, and subsequently extracted and quantified for human DNA, averaging **3.9615 ng/uL at t=0 hours** with five replicates.

Deposition slides that were submerged in both stagnant and flowing water had a drastic drop in DNA quantity at t=6 hours. Under both stagnant and flowing conditions, an increase in DNA quantity was seen (for stagnant, at t=24 hours and for flowing, at t=48 hours) followed by a decrease in DNA quantity (for stagnant, at t=48 hours and for flowing, at t=72 hours) that was then consistent with the DNA quantity recovered at t=168 hours (1 week).

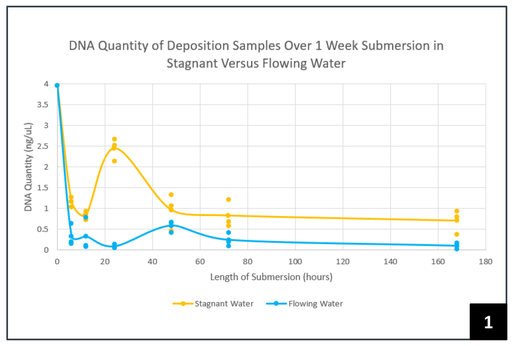
Blank slides that were submerged alongside the deposition slides in stagnant and flowing water had an increase in DNA quantity (for stagnant, at t=24 hours and for flowing, at t=6 hours) followed by a decrease in DNA quantity (for stagnant, at t=48 hours and for flowing, at t=12 hours). From t=72 hours to t=168 hours, the blank in the stagnant water increased in DNA at a higher rate than in the flowing water.

Peak Height Ratio

STR amplification was performed on the highest quantity samples for each time period, in addition to a reference donor sample. **Six loci** where the donor had heterozygous alleles were examined for each sample (D3S1358, vWA, D8S1179, D18S51, D2S441, and FGA). There was no visible trend in peak height ratios among the alleles in either condition, so individual allele peak height was examined instead.

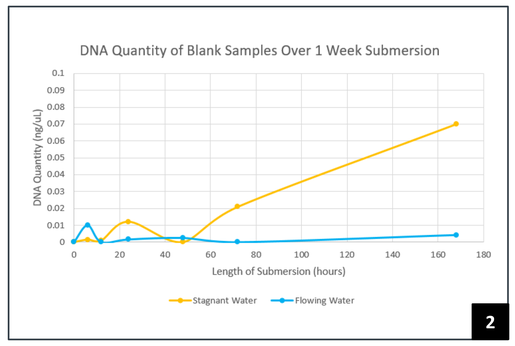
Allele Peak Heights

In general, individual allele peak heights increased and decreased in tandem with one another over time *(note: scan the QR code at the bottom right corner of this poster for more information)*. Under all conditions except the blank samples in flowing water, the peak heights of D8S1179 alleles were highest after 1 week submersion. The variation in allele peak heights appeared to increase over time for deposition and blank samples in flowing water and blank samples in stagnant water. All alleles recovered were consistent with the donor, except in four blank samples and two deposition samples submerged in flowing water *(see limitations for more information)*.



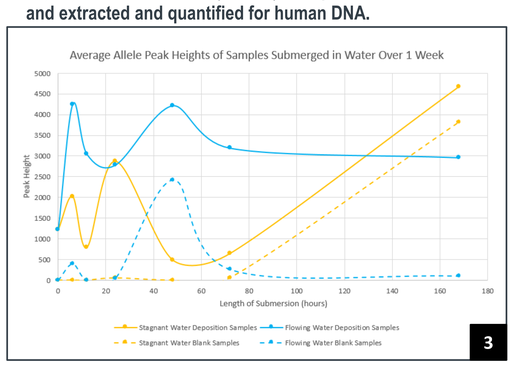
1

Deposition samples were submerged in stagnant or flowing water and removed from the water at time intervals t=6, 12, 24, 48, 72 and 168 hours (1 week). Samples were swabbed and extracted and quantified for human DNA.



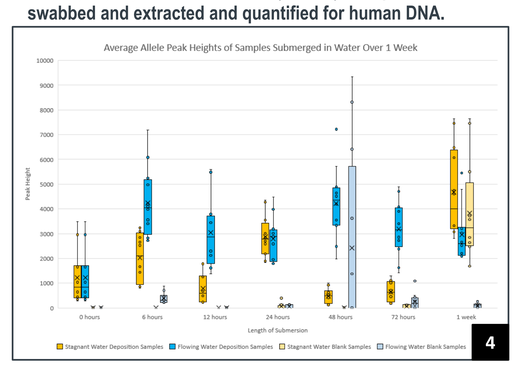
2

Blank samples were submerged with deposition samples in stagnant or flowing water and removed from the water at t=6, 12, 24, 48, 72 and 168 hours (1 week). Samples were swabbed and extracted and quantified for human DNA.



3

STR amplification was performed on deposition and blank samples of the highest quantity per time interval. Six loci with heterozygous alleles were examined. The peak heights of these alleles were averaged for simpler viewing.



4

STR amplification was performed on deposition and blank samples of the highest quantity per time interval. Six loci with heterozygous alleles were examined. The average and distribution of these peak heights was observed.

5. Conclusions

DNA Quantity and Allele Drop-Out Over Time

We hypothesized that DNA quantity and the number of alleles recovered would decrease over time. Although increases in DNA quantity were observed for some time intervals for deposition samples, overall, **DNA quantities did decrease over time in deposition samples submerged in both stagnant and flowing water.** However, complete allele drop-out (at the six loci examined) was not observed in any of the deposition samples exposed to either stagnant or flowing water.

We also hypothesized that DNA quantity and allele drop-out would decrease at a slower rate in stagnant water than in flowing water. In general, deposition samples submerged in stagnant water yielded higher DNA quantities than those in flowing water. **However, deposition samples submerged in flowing water yielded higher allele peak heights, on average, until the t=168 hour (1 week) time interval.**

Correlation Between DNA Quantity and Allele Peak Height

Although there are some similarities in the overall trend of increases and decreases of DNA quantity and allele peak heights, the two are not perfectly correlated. For instance, in stagnant water, deposition samples had a slight decrease in DNA quantity from t=72 hours (rate=-0.00266 ng/uL per hour) to t=168 hours, but allele peak heights had a great increase during this time (rate=42.01042 RFU/hour). Although the lowest average DNA quantity for stagnant deposition samples was seen at t=168 hours, at this time interval, the highest allele peak height was observed. **This suggests that low quantity samples can still yield high peak heights and should still be considered for STR amplification.**

Under all conditions except the blank samples in flowing water, the peak heights of D8S1179 alleles were highest at t=168 hours. D8S1179 is a simple repeat, but other STRs with simple repeats were also examined, (D3S1358, D18S51, and D2S441), so further research could be conducted into the nature of these STRs to suggest why D8S1179 had the highest peak heights.

Allele Drop-In Over Time

Possible DNA transfer was considered by evaluating allele drop-in over time. For at least one time interval, an increase in allele height was seen for deposition and blank samples under both water conditions. For deposition and blank samples submerged in flowing water, the average peak heights increased from 24 hours to 48 hours, indicating allele-drop in during this time, before greatly decreasing between 48 hours and 72 hours and then gradually decreasing to 168 hours. Under stagnant conditions, peak heights gradually increased from 48 to 72 hours and then greatly increased to their maximum values between 72 and 168 hours.

We believe that increases in both quantity and peak height were observed as a result of DNA transfer. A subset of blank slides were swabbed then extracted and quantified for human DNA before submersion to verify the lack of background DNA (values undefined). In most cases, allele drop-in was fully consistent with the donor, so any alleles recovered on the blanks were thought to be a result of transfer during submersion.

All samples (both deposition and blanks) entered their respective water vessels at t=0 and were removed at their assigned time intervals. As deposition samples entered the water, the DNA that had been deposited on the samples may have left the slides and entered the water. Some of this DNA may have been hydrolyzed, while the remaining DNA may have been floating in the water. Under stagnant conditions, this DNA may have eventually settled onto existing deposition samples or blank samples. Because the water was not circulating, the transferred DNA may not have been forced off the slide, accounting for high peak heights after 1 week submersion. Under flowing conditions, when DNA left deposition samples, the moving water may have prevented transferred DNA from settling as easily onto existing deposition samples or blanks.

Overall, variation in DNA quantity and peak heights was observed after exposing samples to stagnant and flowing water conditions.

6. Potential for Impact

Do Submerged Samples Hold Evidentiary Value?

Results from this experiment suggest that **the evidentiary value of a non-porous item should not be discounted simply because it has been submerged in water**, whether in stagnant or flowing conditions. Although DNA quantities and allele peak heights increased and decreased over the course of a week, complete allele drop-out at any of the six loci examined was not seen for any of the deposition samples submerged in either stagnant or flowing water. Additionally, low DNA quantity samples may yield high allele peak heights, so low quantity samples exposed to environmental conditions should still be considered for STR amplification.

Can Secondary DNA Transfer Occur Through Water as a Medium?

The increase in DNA quantity and allele drop-in onto blank samples over time suggests that **DNA transfer may occur through water as a medium.** Investigators should consider that DNA recovered from submerged non-porous items could be a result of secondary DNA transfer from nearby submerged items, rather than someone coming into direct contact with that non-porous item prior to submersion.

7. Limitations

Sample Size

Due to time and budget constraints, we were unable to run as many samples as desired. In an ideal world, we would have liked to perform STR amplification on every blank and deposition sample. We will be increasing our sample size, ideally to eight samples per time period, to obtain a better understanding of any trends and variation in DNA quantity over time.

Water Sources

Additionally, only spring water was utilized as a water source in this study. This was an attempt to control the composition of background materials in the water source, as companies that sell spring water list the average ionic components in their products. However, we would like to see how these results may differ in other water sources including tap water, saltwater, and various natural environments.

Flowing Water Vessel

The vessel containing flowing water was created using PVC pipe, and due to the size of the vessel, it could not be placed under a PCR hood to limit extraneous contamination, which could account for some additional alleles, in addition to donor alleles, being seen.

8. Next Steps

Phase I and I I

The experiments highlighted here represent Phase I and II of this graduate thesis research project. **Phase I** includes exposing blank and deposition samples from one donor to stagnant spring water over one week, and **Phase II** includes exposing blank and deposition samples from one donor to flowing spring water over one week. Further replicates for Phase I and II are being conducted, hopefully ending with at least eight deposition sample replicates and three blank sample replicates per time frame.

Phase III and IV

Phase III and IV of this graduate thesis research project are currently in the works. **Phase III** includes exposing blank and deposition samples from two different donors to stagnant spring water over one week. **Phase IV** includes exposing blank and deposition samples from two different donors to flowing spring water over one week.

Because Phase I and II have shown the possibility of DNA transfer through water as a medium, Phase III and Phase IV will include submerging DNA from two different donors into the same water vessel. After one week, if DNA transfer still occurs, researchers are anticipating to recover mixed profiles on the blank samples and perhaps the deposition samples as well.

References and Suggested Citation

[1] Becker, R.F. (2013). *Underwater Forensic Investigation, 2nd ed.* Boca Raton, FL: Taylor & Francis Group.

[2] Borde, Y. M., Tonnany, M. B., & Champod, C. (2008). A study on the effects of immersion in river water and seawater on blood, saliva, and sperm placed on objects mimicking crime scene exhibits. *Journal of the Canadian Society of Forensic Science*, *41*(3), 149–163. https://doi.org/10.1080/00085030.2008.10757172

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More Information

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