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# Detection And Str Profiling Of Saliva Remaining In Water Bottles

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# DETECTION AND STR PROFILING OF SALIVA REMAINING IN WATER BOTTLES

by

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FORENSIC SCIENCE

## ABSTRACT

As instruments for detecting DNA have become more sensitive and reagents for amplifying DNA become more effective, forensic DNA analysts have obtained short tandem repeat (STR) profiles from evidence containing a small amount of DNA. Sources, such as dandruff, sweat, and saliva, which were once thought to be inadequate for STR profiling, can now be analyzed to effectively establish a profile. These low quantities of DNA can be recovered from liquid samples, such as saliva, or extracted and concentrated from large volumes of buffer, then amplified to give a DNA profile. This study focused on recovering saliva from drinking containers. When drinking from a container, a person leaves epithelial cells on the top of the container. A person also transfers epithelial cells to the inside, bottom of the container.

Currently, the method for testing drinking containers, such as a bottled water, is to swab the top of the bottle and analyze the swabs for DNA. The tops of bottles are exposed, leading to the possibility that a person may contaminate or destroy any epithelial cells that remain on the top of a bottle. The bottom of a bottle has more protection from the outside environment, and there could be more DNA recovered from the bottom of the bottle than the top. For this study, water bottles collected after consumption by volunteers were swabbed at the top to collect saliva, and saliva in the remaining water was collected from inside the bottom. The goal was to compare the

established method of swabbing drinking containers to a new method that was formed in this study and determine which method is better to use in a forensic crime lab. Different methods for collecting DNA from the liquid remaining in drinking containers were also evaluated.

The results of this study indicate that evaporation is a more effective method of concentrating DNA than pelleting cells. Both evaporation methods yielded higher amounts of amplified alleles than pelleting cells. Of the two evaporation methods evaluated, the nitrogen evaporator with a water bath was a better method than the oven. The nitrogen evaporator dried samples faster than the oven.

The findings of this study also show that collecting DNA with lysis buffer is more effective than swabbing. Collection with lysis buffer is better when processing plastic bottles and eliminates a step in extraction. When evaluating the four combinations of concentrating and collecting DNA, the nitrogen evaporator with lysis buffer is the best method as the samples dry faster and an extraction step is eliminated.

When evaluating the samples collected from volunteers, the results show that as the remaining water in the bottles decreased, the DNA left in the liquid also decreased. The samples with the 40 mL of liquid remaining contain the highest average amount of DNA and result in the largest amount of alleles amplified. For comparison to the established method of swabbing the tops of plastic bottles, the findings show that both methods contain similar amounts of DNA. The established method results in the highest amount of alleles amplified. These results show that the current method of processing bottles is better, but targeting the backwash in bottles is viable option for forensic biologists to consider.

Keywords: backwash, saliva, STR profiling

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## DEDICATION

To my family,

Throughout my endeavors, your support never wavered.

In our family, sarcasm is a must with patience as a secondary.

Because for us, life without humor is really no fun at all.

The jokes and puns are our form of love.

Even the absolutely awful ones that always seem to make everyone crack a smile.

We are perfectly imperfect.

And, that's just one thing about us that I love.

Because sometimes my scholarly explanations make complete nonsense.

And, sometimes that doesn't really matter at all.

It's your encouragement that shines through.

Especially after I successfully reattempt the explanation.

We may be separated states apart or even across the world.

But, distance does not matter.

For our love, kindness, and warmth always find a way to close the space and fill my heart with overflowing happiness.

No number of "thank yous" will ever be enough.

Instead, I will always strive to be the best daughter, sister, niece, cousin, and friend.

And, I will always try to make you proud.

## ACKNOWLEDGEMENTS

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Thank you for all of your wisdom and recommendations as they were very encouraging to me during my research and graduate studies.

Without the support of my family, friends, and professors, my education and this research would not have been possible.

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CHAPTER 1

# INTRODUCTION

Advances in the technology of forensic biology and their application in criminal cases have allowed identifications to be made from once unlikely sources of DNA. These unlikely sources are pieces of evidence, which are collected at crime scenes and contain minute amounts of biological material, often hidden to the naked eye. In order to properly identify items as evidence and obtain DNA profiles from these sources, determining the location of the biological material on evidence and developing an effective method to collect all the DNA is necessary.

Drinking containers left at crime scenes may become evidence due to their potential as a source for fingerprints or DNA. The location of epithelial cells on a drinking container cannot be seen with the naked eye. Investigators may assume cellular material is located at the top of the bottle where the mouth makes contact. It is possible that DNA is also present in the bottom of drinking containers. Developing a method to recover DNA from the bottom of drinking containers is the purpose of this study.

## *Small Quantities of DNA on Evidence*

Prior to the availability of methods to amplify DNA, forensic labs were able to analyze restriction fragment polymorphisms from blood, semen, and buccal swab samples containing DNA of high quality and quantity. Blood and semen samples were commonly collected by crime scene analysts, especially when the samples were clearly visible at a crime scene. As amplification of DNA was validated and accepted as an analysis method, crime scene analysts were able to analyze DNA from blood and semen

stains that were small, old, or damaged. Other biological samples, such as sweat stains from clothing [1], bones [2], hair [3], or fingernails [4], became viable sources of DNA. In additional to biological stains and samples, DNA has been recovered from other pieces of evidence that made brief contact with a person, such as lipstick [5], cheese [6], cigarette butts [7], and bottles [8]. These sources contained very small amounts of DNA that at one time were too small to elicit a DNA profile. With this progression, small amounts of DNA, which by standard means would not be adequate for testing, yielded DNA profiles.

The sensitivity of amplification and analysis has improved by modifying the methods. In 2004, Parys-Proszek studied improving microsatellite analysis in samples with low copy numbers of DNA [9]. The analysis was adjusted by adding more template DNA purifying PCR products with cellulose and silica membranes, adding more PCR product, allowing a longer time duration of injection during capillary electrophoresis, or increasing the number of PCR reaction cycles. Of these methods that address low copy number DNA, Parys-Proszek focused on obtaining profiles by adding cycles during amplification. Although the use of these small amounts is questionable for use in court, the DNA in these small samples produced profiles.

New methods allowed for analysis of the DNA transferred from a person to an item through touch. Djuric et al. amplified DNA extracts from touched objects using a 34-cycle PCR [10]. The focus of the study was to determine the effect of time had on the quality of the DNA recovered from plastic tubes. The DNA in seven samples produced full profiles despite the low quantities of DNA recovered from the plastic tubes.

Daly et al. also focused on obtaining STR profiles from touch DNA left on specific objects [11]. This study had 300 participants hold wood, glass, and cloth. The objects were sterilized before any handling to eliminate any DNA on the objects. One hundred and fifty men and 150 women touched and held a piece of wood, glass, or fabric for 60 seconds. The objects were swabbed, and the swabs were extracted. The objects were all quantified, but only a portion of the samples was profiled. For the glass, one of the 40 samples picked for profiling produced a full profile. Of the remaining samples, 19 glass objects produced no profiles and 20 glass objects produced partial or mixed profiles. For the fabric, 14 of the 24 samples picked for profiling produced full profiles. The remaining eight samples produced mixed or partial profiles. For the wood, 12 of the 59 samples gave full profiles. Of the remaining samples, seven wood objects produced no profiles and 40 wood objects produced mixed or partial profiles. The average amount of DNA recovered from the objects was 5.85 ng for the wood, 1.23 ng for the fabric, and 0.52 ng for the glass.

*Evidence with Trace Amounts of Saliva*

For saliva, objects that have been touched by the lips or mouth, such as glass or a piece of food, are inferred to contain cheek cells from saliva. DNA has been recovered from small amounts of cheek cells in saliva which have been transferred to a variety of objects collected as evidence. In one case report, Sweet et al. documented a DNA profile being given from saliva that was swabbed from a cheese bite [6]. The cheese yielded a DNA profile after proper storage of the cheese and from swabbing the bite marks where the lips and tongue would have touched the cheese.

Lip cosmetics have also been studied as a source for DNA [5]. Webb et al. swabbed and tested 38 lip cosmetics, containing 25 different brands and types of lip cosmetics, from 11 different participants. The used surface of each lip cosmetic was swabbed to collect as much saliva as possible. The samples were tested by attempting amplification of 10 STR loci, the standard number of STR loci analyzed in Australia, where the study was done. The samples yielded 17 profiles with 10 STR loci, 14 partial profiles, and seven samples that did not give a profile. Some partial profiles contained a mixture of DNA, which could be attributed to sharing the lip cosmetic or kissing someone. Despite the small area where saliva is deposited, it was suggested that the repeated contact with the cosmetic leads to the presence of enough DNA for successful amplification.

*Locating Trace Amounts of Saliva on Evidence*

During collection, locating sources of small amounts of DNA is not possible with the naked eye. For traces of blood or semen, stains not visible in ambient light can be located on evidence with a chemiluminescent reaction or an alternate light source (ALS). Dandruff and dead skin may be overlooked because of the small size and fragility. By brushing items of clothing, analysts can find hidden loose hairs and dead skin cells that possibly contain DNA. Another common source of DNA is sweat, because somatic cells are present in sweat. Like blood and semen, sweat can be found using an alternative light source since sweat also fluoresces with an ALS. Because sweat comes from the pores of the body, sweat can be found in a variety of places, including clothing, bedding, and fingerprints. Locating evidence is difficult when sources of DNA are not in plain view.

Often items are collected as possible sources of DNA evidence, but whether the evidence actually contains DNA was not immediately evident [12].

Determining the location of DNA on a piece of evidence where residual saliva is present can be resolved by using quick and simple field tests. Two common methods for locating saliva are the Phadebas test and alternate light source [13]. In the presence of saliva, the Phadebas test is turned blue. Specifically, the amylase in saliva reacts with Phadebas paper to turn the paper blue. However, saliva is not always detected by the Phadebas paper. An ALS is also used to locate saliva. When exposed to alternate light, saliva is detected by the fluorescence, but, like the Phadebas paper, saliva is not always discovered by an ALS.

*Collecting Saliva from Bottles*

A common source of saliva for DNA analysis has been on the top of plastic bottles. A person takes a sip from his drink and transfers epithelial cells from his mouth to the top of the plastic bottle. The epithelial cells remain on top of the bottle after use and persist unless intentionally wiped off the container. When the bottle is collected and stored properly, cells from the saliva remain on the bottle and can result in a DNA profile. Hedman et al. replicated bottles collected from crime scenes by distributing 0.5, 2, 8, and 32 microliters of saliva around the top of 12 plastic bottles [14]. Also, one participant drank from three different bottles of mineral water. Saliva was collected from the tops of the bottles and tested for a DNA profile. Full profiles were obtained from the six plastic bottles with 8 and 32 microliters of saliva on their tops. Partial profiles were attained from two of the three bottles with 2 microliters of saliva on their tops and the three plastic bottles that the participant consumed. Hedman et al. determined that no

profile was obtained from the three drinking containers with 0.5 microliters of saliva because the amount of saliva was too low to yield a DNA profile.

A study by Abaz et al. focused on obtaining a DNA profile from saliva collected from bottles and cans containing different types of liquids, including water, soda, alcohol, or milk [8]. Abaz et al. observed the variation in saliva production between individuals, the effect certain types of liquid had on DNA recovery, the time allowed for DNA to dry on the drinking container, and the effect the type of container had on DNA yield. Six participants consumed 96 samples of various drinks in bottles and cans, and the saliva was tested for 10 STR loci and the Amelogenin locus, the standard for New Zealand. In this study, the 96 samples produced 73 full profiles, 10 partial profiles, and 13 that failed to give a profile. From these samples, non-alcoholic drinks resulted in more DNA recovered and had higher peak intensities, and the alcoholic drinks were more likely to cause a decline in DNA yield and quality. Both the Hedmen et al. and Abaz et al. studies targeted drinking containers that yielded DNA profiles. Like cosmetics, the surface area at the top of the drinking container is small, but enough DNA accumulated on this area to result in successful amplification.

Although the established method for collecting saliva from drinking containers is to swab the top of the container with a moistened swab, the bottom of drinking containers may contain more DNA, under some circumstances [15]. The top area of a drinking container has a small surface for saliva to adhere. A swab is moistened and rubbed around the drinking container to collect as much of the saliva as possible. However, some of the saliva on the top of the drinking container may be transferred to the lid, if the lid is repeatedly placed on and taken off the container. Additionally, the saliva may be wiped

off accidentally or purposefully when the lid is off, and the outside of the top is exposed to the environment. When a person is drinking a beverage, saliva is likely to be transferred to the liquid inside the container. The saliva in the liquid will remain in the bottom of the drinking container, protected from the outside environment. The cheek cells within the saliva could contain enough DNA to be extracted and amplified, resulting in a DNA profile. Also, unlike the top of the container, the saliva in the bottom of the container cannot be easily removed. Targeting a large volume of liquid containing a small amount of cheek cells does present a challenge, but previous research suggests recovering DNA from a liquid sample containing cheek cells is possible.

When targeting DNA located in the bottom of drinking containers, several variables exist that can affect the yield and quality of DNA. Different container materials may bind cells or DNA differently, which can affect the quantity of the DNA collected. The type of drink could also have an effect on the amount of DNA collected. More important than the type of container or liquid is the amount of saliva and liquid left in the container, which will likely vary from drink to drink as well as person to person. A person may consume the drink all at once, which leaves little saliva on the container, or ingest the drink at a constant rate periodically throughout the day until the drink is gone. An increase in the number of sips taken from the drink should increase the amount of saliva left in the container. The way a person drinks would also affect the amount of saliva left on and in the container. People salivate at different rates, and one person may put more saliva into the liquid with one sip than another person. Regardless of the amount of saliva in the container, any remaining liquid will dilute the saliva, making recovery more difficult. Evaporation can also affect the ratio of liquid to saliva. The liquid could

evaporate and concentrate the saliva at the bottom, resulting in a more concentrated sample.

*Methods of Concentrating Epithelial Cells in Diluted Sources*

Although recovering DNA from the liquid in drinking containers has not been attempted, recovering DNA from saliva diluted in large volumes of liquid has been successfully demonstrated. In a study by Johnson, diluted semen, diluted blood, and diluted saliva soaked in buffer were concentrated through the use of a vacuum filtration system, producing enough DNA for analysis [16]. A vacuum filtration method was established and compared to an evaporation method. For each method, 30, 10, 5, and 1 microliters of saliva, blood, and semen, each in 250 milliliters of TE, Gill, DI $H_2O$, or Tween-80 buffers were analyzed. In the evaporation method, the samples were incubated for three days at 70°C, which reduced the sample to 50 milliliters. Compared to the evaporation method, the vacuum filtration method yielded significantly higher amounts of DNA in the diluted samples of saliva, blood, and semen. For saliva samples with the vacuum filtration method, Johnson reported DI $H_2O$ buffered samples giving the highest concentration of 6 ± 2 ng/µL. The lowest concentrations of DNA for saliva were 0.3 ± 0.3 ng/µL in the Tween-80 buffer. This study focused on quantitating small volumes of DNA from large volumes of liquid. Profiles were not obtained from a STR profile analysis of the DNA. The quantities resulted from the study were in large enough amounts to give a STR profile.

Urine is another example of a sample that contained small amounts of DNA from epithelial cells. A study by Shan et al. focused on concentrating DNA from urine samples using carboxylated magnetic nanoparticles [17]. The DNA was not quantitated or used to

produce a DNA profile. The authors used gel electrophoresis to determine the amount of DNA extracted from the urine sample. The brightness of the band on the gel electrophoresis determined the concentration of the DNA. Higher concentrations of DNA produced a brighter band on the gel electrophoresis. The DNA was concentrated, and the amount was likely able to yield a profile. But, the analysts decided to not pursue quantitation or identification.

Although skeletal remains are not discovered as a liquid sample, skeletal remains can be soaked in a large volume of lysis buffer to extract DNA from the bones [2]. Skeletal remains contain low quantities and qualities of DNA, and the risk of not getting a DNA profile from the bones is often the reason for using bones as a last resort DNA source. When attempted, pulverizing the center of bones into dust results in more DNA being released. The study by Alonso et al. attempted amplification and identification of DNA from bone samples found in mass graves and forensic bone and teeth samples obtained from casework using multiplex STR systems and megaplex STR systems [2]. Samples included 126 blood or bloodstain reference samples, 10 DNA extracts from teeth samples, and 76 DNA extracts from bone samples. Amplifications of the bone dust and teeth samples were performed using PowerPlex 16 System, AmpfISTR Profiler PCR Amplification Kit, AmpfISTR Profiler Plus Amplification Kit, or AmpfISTR COfiler Amplification Kit. STR profiling showed that DNA in bone samples amplified to give full and partial profiles although some samples failed to amplify. Alonso et al. noted that the long human bones gave a higher quality of DNA when compared to DNA extracted from skulls or ribs.

Rogers et al. reports a median yield of 55 micrograms of DNA from the saliva per 10 milliliters of Scope Original Mint mouthwash [18]. The participants rinsed the mouthwash vigorously to extract a large amount of cheek cells. While generating a DNA profile was not part of the study, 55 micrograms of DNA per 10 milliliters of mouthwash is an adequate amount of DNA to produce a DNA profile. Since the DNA was not destroyed by the mouthwash, collecting DNA from other types of liquid found in the bottom of the drinking containers seems possible.

*Recovering DNA from the Bottom of Drinking Containers*

This study focuses on obtaining a DNA profile from water left in the bottom of drinking containers. This study will compare the current method of collecting DNA from the top of drinking containers to the proposed method of collecting DNA from the inside bottom of drinking containers. The volume of water consumed will be varied, resulting in different volumes of water left in the bottom of the drinking containers. The goal is to compare the new method that will be established in this study against the method currently used in the forensic community and determine which method is better to use in a forensic crime lab.

CHAPTER 2

# COMPARISON OF METHODS FOR COLLECTING DNA FROM BOTTLED WATER SPIKED WITH SALIVA

Two DNA concentration methods, pelleting cells and evaporation, were assessed using a centrifuge, an oven, and a nitrogen evaporator with a water bath in order to determine the most efficient method for concentrating and collecting any epithelial cells left in liquid samples.

## Methods & Materials

Three methods for concentrating and collecting DNA from the bottom of water bottles were compared: drying the bottles in an oven, pelleting cells in water removed from the bottles using a centrifuge, and evaporating water removed from the bottles under nitrogen.

### *Sample Preparation*

Saliva was collected by having the principal investigator spit into a glass vial. All samples were prepared by spiking water with collected saliva.

Eighteen bottles of Dasani water (The Coca-Cola Company, Atlanta, GA) were emptied, and 20 mL of the bottled water was pipetted back into each bottle. To each bottle, 250 µL of saliva were added. The bottles were shaken and left at room temperature for an hour. Each bottle was then cut in half for the DNA concentration step.

Six bottles were dried in a Blue M 11W Single Wall Transite Gravity Convection Oven at 70°C. The samples in the oven were checked every hour until the bottles were completely dried, approximately six hours and 30 minutes. The bottom of the dried bottle was swabbed with one moistened cotton swab, and the swab was placed into a 1.5 mL tube for extraction.

Cells were pelleted for six samples buy pipetting the water and saliva mixture into 16x25 mm glass test tubes, with each 20 mL sample divided into two tubes with 10 mL each. Samples were placed in the DYNAC Centrifuge (Clay Adams Dickinson & Company, Parsippany, NJ) and spun for five minutes on full speed to concentrate the cells at the bottom of the tube. The supernatant was pipetted out of each tube, leaving any visible cell pellet and approximately 700 µL of water. The residues in the centrifuge tubes were pipetted into 1.5 mL tubes for extraction. The bottom of the dried bottle and the dried test tubes were swabbed with one moistened cotton swab, which was also added to the extraction tube.

The remaining six samples were dried under nitrogen by first transferring the water and saliva mixture into 16x25 mm glass test tubes, with each 20 mL sample split into two tubes, each containing 10 mL of liquid. These sample tubes were placed in an OA-SYS Heating System and N-EVAP™ 112 Nitrogen Evaporator (Organomation Associates, Inc., Berlin, MA). The heating system of the water bath was set to high, and the flow rate of the nitrogen gas from the tank to the evaporator was adjusted to keep the pressure of flow at 20 psi. The samples in the nitrogen water bath were checked every 30 minutes, adjusting the needles that blew nitrogen into each tube, until the samples were completely dried, approximately two hours and 30 minutes. For each sample, the bottom

of the dried bottle and the dried test tubes were swabbed with one moistened cotton swab, and the swab was placed in a 1.5 mL tube for extraction.

For extraction controls, two 250 µL samples of saliva were pipetted into separate 1.5 mL tubes for positive controls, and two 250 µL aliquots of water were pipetted into separate 1.5 mL tubes for negative controls.

*Extraction*

DNA was extracted from the samples using the Qiagen DNA Investigator Kit (Qiagen, Valencia, CA) and a modified protocol. After addition of 300 µL ATL lysis buffer and 20 µL proteinase K to each sample and control, the swabs in the 1.5 mL tubes were incubated in a water bath overnight at 56°C. Following incubation, 300 µL AL buffer were added to each tube, which was then vortexed, and incubated for 10 minutes in the water bath at 70°C. Clean tweezers were used to pull the swabs from the tubes, a spin basket was inserted into each tube, and the swabs were placed into the spin baskets. The tubes were spun in a Shelton Scientific VSMC-13 Mini-Centrifuge at full speed for one minute. After centrifuging, the swabs and spin baskets were discarded. To each sample, 150 µL of 98% ethanol were added and vortexed before the mixture was pipetted into a labeled spin column. The mixtures in the spin columns were centrifuged at 3309 x g (8000 rpm) for one minute. The flow-through was discarded, and the spin columns were placed back in the collection tubes. To each sample, 500 µL Buffer AW1 were added, and the spin columns were centrifuged for one minute at 3309 x g (8000 rpm). The flow-through was discarded, and the spin columns were placed back into the collection tubes. To each sample, 500 µL Buffer AW2 were added, and the spin columns were centrifuged for one minute at 3309 x g (8000 rpm). The flow-through was

discarded, and the spin columns were placed into clean collection tubes. To each sample, 500 µL of 98% ethanol were added, and the spin columns were centrifuged for three minutes at 10,135 x g (14,000 rpm). The flow-through and collection tubes were discarded, and the spin columns were placed into labeled 1.5 mL tubes. To each sample, 100 µL Buffer ATE were pipetted directly onto each DNeasy membrane of the DNeasy Mini spin columns, incubated at room temperature for one minute, and centrifuged for one minute at full speed to elute. The final elution volume for each sample was 100 µL.

*Amplification and Analysis*

The extracted DNA was amplified using a customized three loci multiplex targeting D3S1358, TH01, and D13S317. The locus D3S1358 is located on chromosome 3p21.31. Locus D3S12358 is a STR locus within the intergenic DNA on chromosome 3. This locus is a single copy in the genome and is the locus number 1358 described on chromosome 3. The locus TH01 is a STR locus located on chromosome 11p15.5 in the first intron of tyrosine hydroxylase gene. The locus D13S317 is located on chromosome 13q31.1. Locus D13S317 is a STR locus within the intergenic DNA on chromosome 13. This locus is a single copy in the genome and is the locus number 317 described on chromosome 13. The three STR loci were targeted using five pmol of each of the following primers:

D3S1358 forward primer – 5’-dye-ATGAAATCAACAGAGGCTTGC-3',

D3S1358 reverse primer – 5'-ACTGCAGTCCAATCTGGGT-3',

TH01 forward primer – 5'-dye-ATGAAATCAACAGAGGCTTGC-3',

TH01 reverse primer – 5'-ATTCAAAGGGTATCTGGGCTCTGG-3',

D13S317 forward primer – 5'-dye-GGCAGCCCAAAAAGACAGA-3',

D13S317 reverse primer – 5'-ATTACAGAAGTCTGGGATGTGGAGGA-3'. Nuclease free water was added to bring the reaction to a total volume of 25 µL. DNA was amplified under the following conditions: denatured for 11 minutes at 95°C, held at 96°C for 1 minute, followed by 10 cycles of 94°C for 30s, 58°C for 30s, 70°C for 45s, then 20 cycles of 90°C for 30s, 58°C for 30s, 70°C for 45s, then held at 60°C for 30 min.

Amplified product was analyzed using Applied Biosystems 310 Genetic Analyzer and GeneMapper® ID Software v3.2 (Applied Biosystems). Amplified product was prepared by combining 1 µL of amplified product with 24.5 µL Fisher BioReagents™ formamide (Fisher Scientific, Pittsburg, PA) and 0.5 µL of GeneScan 500 LIZ Size Standard (Applied Biosystems). When performing analysis, peaks were recorded with a threshold exceeding 50 relative fluorescence units (rfus).

## Results & Discussion

Given the amplification reaction targets three loci, each sample had the potential for successful amplification of six alleles, and each group of six samples had the potential for successful amplification of 36 alleles. In Figure 1, the graph shows the comparison of alleles amplified by the three methods. Samples that were dried in the oven resulted in the amplification of 21 of 36 STR alleles (58%), one full profile, two partial profiles with dropout at D3S1358, and three partial profiles with dropout at D3S1358 and TH01.

Samples that were centrifuged to collect cells resulted in the amplification of three of 36 STR alleles (8%). Of the six samples using the centrifuge, there was one partial profile with dropout at D3S1358 and TH01, one partial profile with dropout at D3S1358, TH01, and D13S317, and four samples that yielded no DNA profiles.

Samples that were evaporated under nitrogen resulted in the amplification of 32 of 36 STR alleles (89%). There were three full profiles and three partial profiles with dropout at D3S1358.

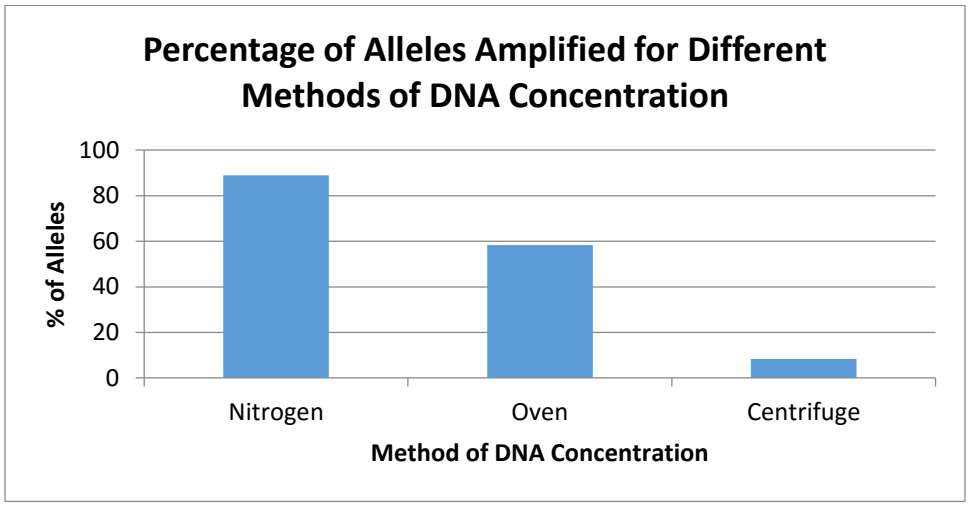


Figure 1. The graph depicts the percentages of alleles amplified using different methods for concentrating DNA from 20 mL water samples spiked with 250 µL of saliva. Samples evaporated under nitrogen (89%) resulted in more alleles being amplified than samples dried in an oven (58%) and samples centrifuged to form a cell pellet (8%).

Drying the samples with a nitrogen evaporator and drying samples in an oven are the preferred methods when compared to centrifuging samples to collect cells. The centrifuge may not have been strong enough to accumulate all the epithelial cells into a pellet. Some of the cells may have remained suspended in the supernatant that was pipetted off and discarded. Also, cells in the sample may have lysed, releasing DNA into the supernatant, resulting in some loss of DNA. The Rogers [18] study centrifuge saliva samples to concentrate epithelial cells, but the DNA from the 10 mL oral rinse samples yielded a much higher amount of DNA, about 55 µg, which would have contained more than enough DNA for a full STR profile. The Rogers study also had volunteers

vigorously swish the oral-rinse solution in their mouths, which could have led to more cells in their samples than were contained in the 250 µL of saliva used in this study. The vigorous swishing of mouthwash may have caused more saliva to shed into the oral-rinse solution as opposed to sipping of water.

The water bath with a nitrogen evaporator and the oven drying methods were the preferred methods for DNA concentration as they produced results similar in quality to the two positive controls of 250 µL saliva (Figures 2 and 3). The amplified alleles from the spiked samples evaporated with nitrogen evaporator had peak heights similar to the positive controls. The results for the oven were also similar quality to the electropherograms of the respective positive controls. The analysis of the two negative controls of 250 µL water showed no alleles.

Between the two drying methods, the nitrogen evaporator produced better results than the oven method. For the oven, the water was not transferred into test tubes, instead opting for a greater surface area for drying. This greater surface area may have indirectly resulted in a loss of DNA. The plastic bottles have small crevices that the liquid dried in but were too small for collection by the swab or the large area was more difficult to completely be swabbed. The test tubes used for the nitrogen evaporator have a smaller, smoother surface. This smaller area may have reduced DNA loss and concentrated the epithelial cells in a much smaller area.

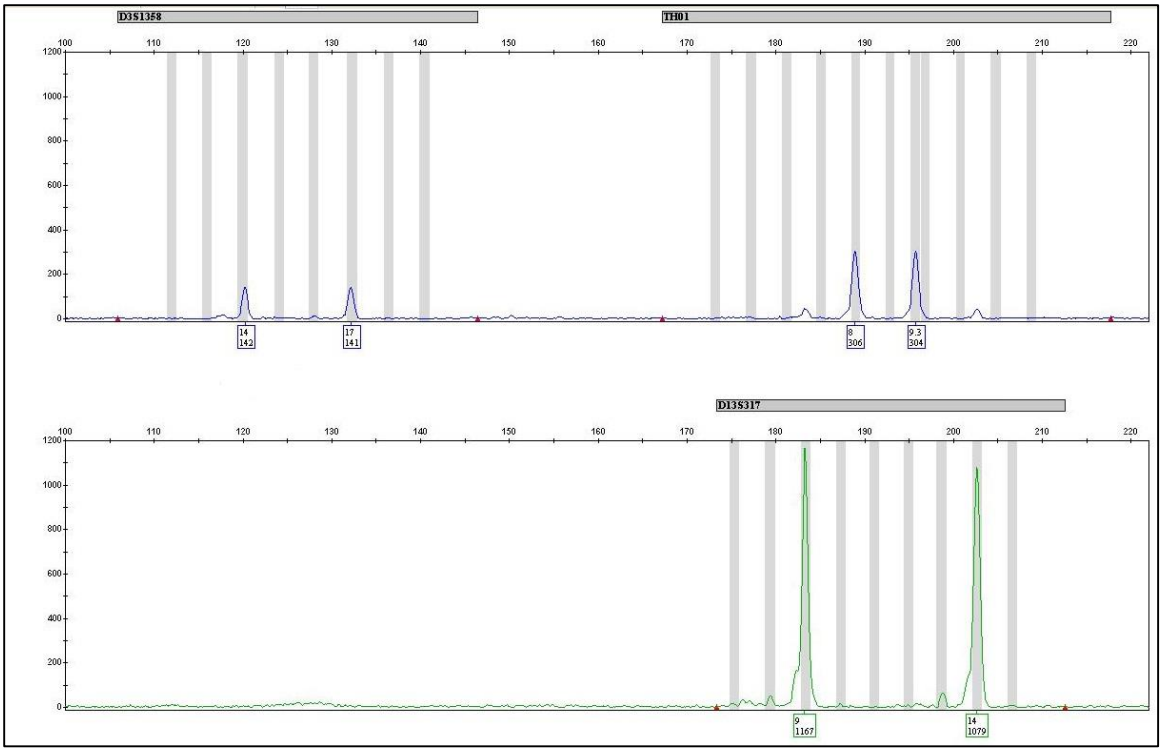


Figure 2. Electropherogram of sample dried with the nitrogen evaporator. The sample was 20 mL of water spiked with 250 µL of saliva, which was removed from the original bottle, separated into glass test tubes, and evaporated under nitrogen. This is one of the three nitrogen-dried samples that resulted in a full profile of six alleles.

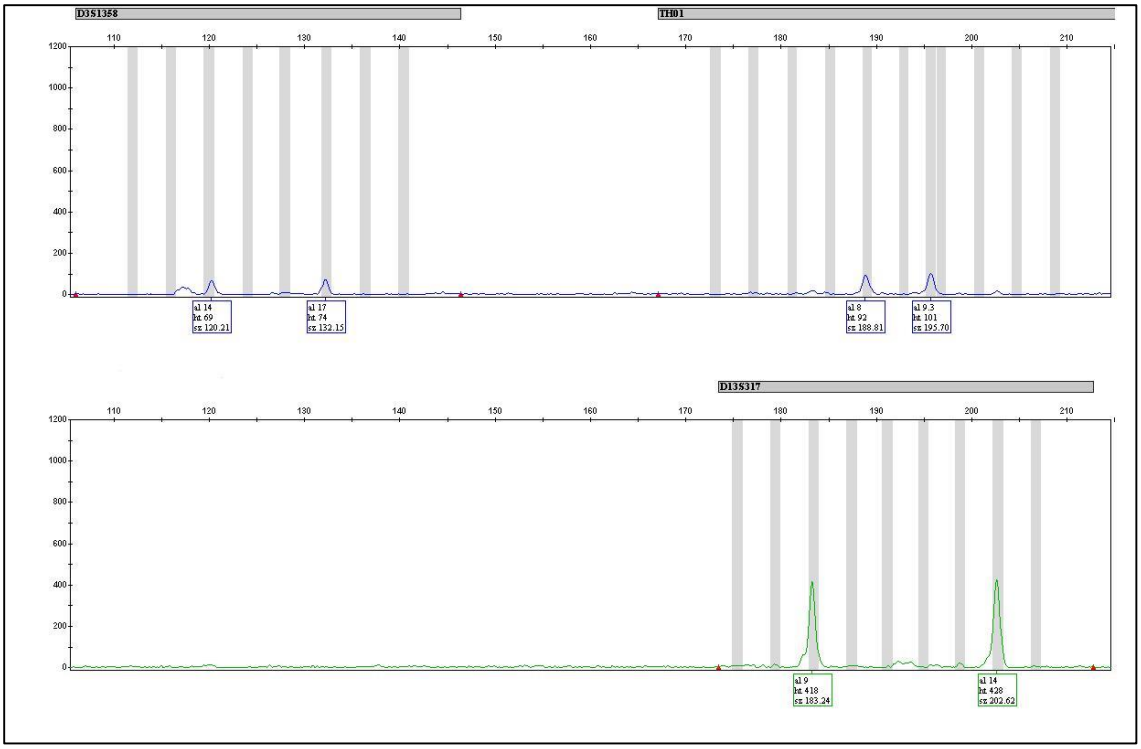


Figure 3. Electropherogram of positive control for extraction. The positive control was 250 µL of saliva pipetted into a 1.5 mL tube. This is one of the two positive controls used for extraction.

CHAPTER 3

# METHOD DEVELOPMENT: EVAPORATING WATER AND 100 µL OF SALIVA IN BOTTLES

In the second step of method development, samples were collected by two different methods: swabbing and pipetting ATL lysis buffer into the bottles and test tubes. Only the oven and the nitrogen evaporator were used for concentrating the samples. A total of four methods were compared: drying in the oven with collection by swabs, drying in oven with collection by ATL lysis buffer, drying in the nitrogen evaporator with collection by swabs, and drying in the nitrogen evaporator with collection by ATL lysis buffer. This step in the method development evaluated effect of the swabs with the dry-down methods, as their elimination would result in a faster and easier extraction.

## Methods & Materials

### *Sample Preparation*

Sample preparation was the same as in the first step of method development. The only change was in the in the DNA collection step.

Six bottles were dried in a Blue M 11W Single Wall Transite Gravity Convection Oven. For three of the samples, 350 µL ATL lysis buffer from the Qiagen DNA Investigator Kit (Valencia, CA) was pipetted into the bottom of the dried bottle. Using the pipette, the buffer solution was swished around the bottom of the container. After

swishing, the solution was pipetted into a 1.5 mL tube. For the remaining three oven-dried samples, the bottom of the dried bottle was swabbed with one moistened cotton swab, and the swab was placed into a 1.5 mL tube for extraction.

The remaining six samples were dried under nitrogen. For three of the samples, 350 µL ATL lysis buffer from the Qiagen DNA Investigator Kit were pipetted into the bottom of the dried bottle. Using the pipette, the buffer solution was swished around the bottom of the dried bottle and transferred from the dried bottle to each of the dried test tubes. After swishing the buffer in the bottle and test tubes, the solution was pipetted into a 1.5 mL tube for extraction. For each of the remaining three nitrogen-dried samples, the bottoms of the dried test tubes were swabbed with one moistened cotton swab, and the swab was placed into a 1.5 mL tube for extraction.

For extraction controls, one 100 µL samples of saliva was pipetted into a 1.5 mL tube for a positive control, and one 100 µL aliquot of water was pipetted into a 1.5 mL tube for a negative control.

*Extraction*

DNA was extracted from the samples and controls using the Qiagen DNA Investigator Kit (Qiagen, Valencia, CA) and a modified protocol. For samples collected using ATL lysis buffer, no additional volume of ATL lysis buffer was added. For swabs and controls, 300 µL of ATL lysis buffer were added to each sample. After addition of 20 µL proteinase K, the samples in the 1.5 mL tubes were incubated in a water bath overnight at 56°C. Following incubation, 300 µL AL buffer was added to each tube, vortexed, and incubated for 10 minutes in the water bath at 70°C. For the samples containing a swab, clean tweezers were used to pull the swabs from the tubes, a spin

basket was inserted into each tube, and the swabs were placed into the spin baskets. The tubes were centrifuged at full speed for one minute. After centrifuging, the swabs and spin baskets were discarded. To each sample, 150 µL of 98% ethanol were added and vortexed before the mixture was pipetted into a labeled spin column. The mixtures in the spin columns were centrifuged at 3309 x g (8000 rpm) for one minute. The flow-through was discarded, and the spin columns were placed back in the collection tubes. To each sample, 500 µL Buffer AW1 were added, and the spin columns were centrifuged for one minute at 3309 x g (8000 rpm). The flow-through was discarded, and the spin columns were placed back into the collection tubes. To each sample, 500 µL Buffer AW2 were added, and the spin columns were centrifuged for one minute at 3309 x g (8000 rpm). The flow-through was discarded, and the spin columns were placed into clean collection tubes. To each sample, 500 µL of 98% ethanol were added, and the spin columns were centrifuged for three minutes at 10,135 x g (14,000 rpm). The flow-through and collection tubes were discarded, and the spin columns were placed into labeled 1.5 mL tubes. To each sample, 100 µL Buffer ATE were pipetted directly onto each DNeasy membrane of the DNeasy Mini spin columns, incubated at room temperature for one minute, and centrifuged for one minute at full speed to elute. The final elution volume for each sample was 100 µL.

*Amplification and Analysis*

The extracted DNA was amplified using a customized three loci multiplex (D3S1358, TH01, and D13S317) and Promega Go Taq® Hot Start Colorless Master Mix (Madison, WI). The reaction was composed of 12.5 µL Promega Go Taq® Hot Start

Colorless Master Mix, 2 µL of extracted DNA, and 5 pmol of each of the following primers:

D3S1358 forward primer – 5’-dye-ATGAAATCAACAGAGGCTTGC-3',

D3S1358 reverse primer – 5'-ACTGCAGTCCAATCTGGGT-3',

TH01 forward primer – 5'-dye-ATGAAATCAACAGAGGCTTGC-3',

TH01 reverse primer – 5'-ATTCAAAGGGTATCTGGGCTCTGG-3',

D13S317 forward primer – 5'-dye-GGCAGCCCAAAAAGACAGA-3',

D13S317 reverse primer – 5'-ATTACAGAAGTCTGGGATGTGGAGGA-3'.

Nuclease free water was added to bring the reaction to a total volume of 25 µL. DNA was amplified under the following conditions: denatured for 11 minutes at 95°C, held at 96°C for 1 minute, followed by 10 cycles of 94°C for 30s, 58°C for 30s, 70°C for 45s, then 20 cycles of 90°C for 30s, 58°C for 30s, 70°C for 45s, then held at 60°C for 30 min.

Amplified product was analyzed using Applied Biosystems 310 Genetic Analyzer and GeneMapper® ID Software v3.2 (Applied Biosystems). Amplified product was prepared by combining 1 µL of amplified product with 24.5 µL Fisher BioReagents™ formamide (Fisher Scientific, Pittsburg, PA) and 0.5 µL of GeneScan 500 LIZ Size Standard (Applied Biosystems). When performing analysis, peaks were recorded with a threshold exceeding 50 rfus.

## Results & Discussion

All samples resulted in full profiles (Figure 4). There were no differences in the percentage of alleles amplified between the water and saliva samples that were dried in

an oven then swabbed, dried in an oven then rinsed with lysis buffer, dried under nitrogen then swabbed, or dried under nitrogen then rinsed with lysis buffer.

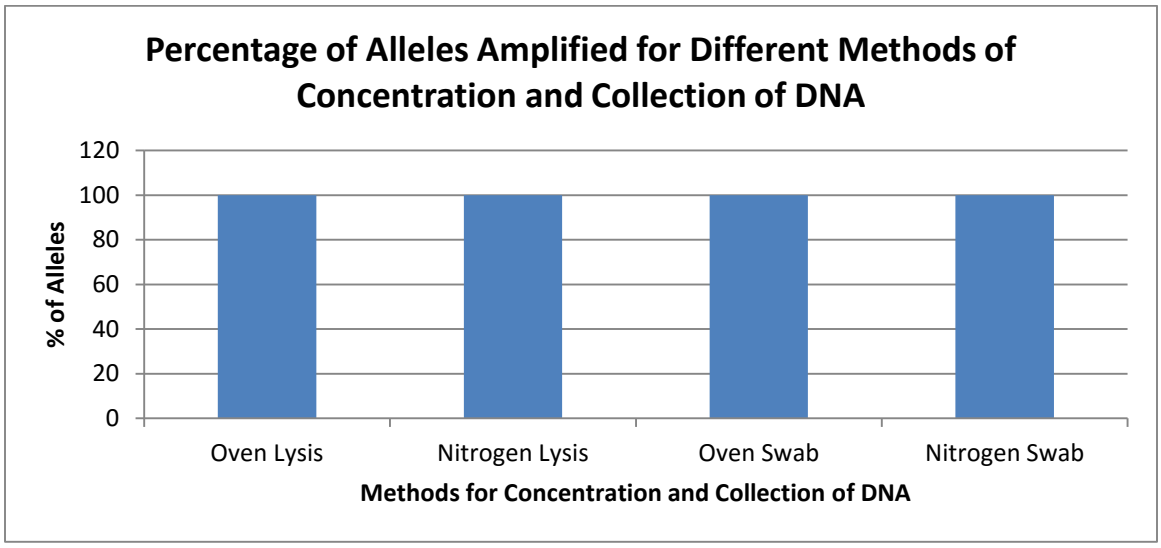


Figure 4. The graph depicts the percentages of alleles amplified using the different methods of concentration and collection of DNA. All methods had 100 percent amplification of alleles.

When time and method simplicity are taken into consideration, drying the samples with the nitrogen evaporator and collecting the residue with lysis buffer was the best combination for concentrating and collecting the samples. Both oven and nitrogen concentration methods yielded results of similar quality (Figure 5), but the nitrogen evaporator required less than half the time to dry, when compared to the oven. Also, as mention in the previous Chapter, DNA collection from the bottles was more difficult, unlike the smaller glass test tubes. Both swab and buffer collection methods provided results with similar peak heights, but collection with the lysis buffer eliminated the spin baskets from the extraction process.

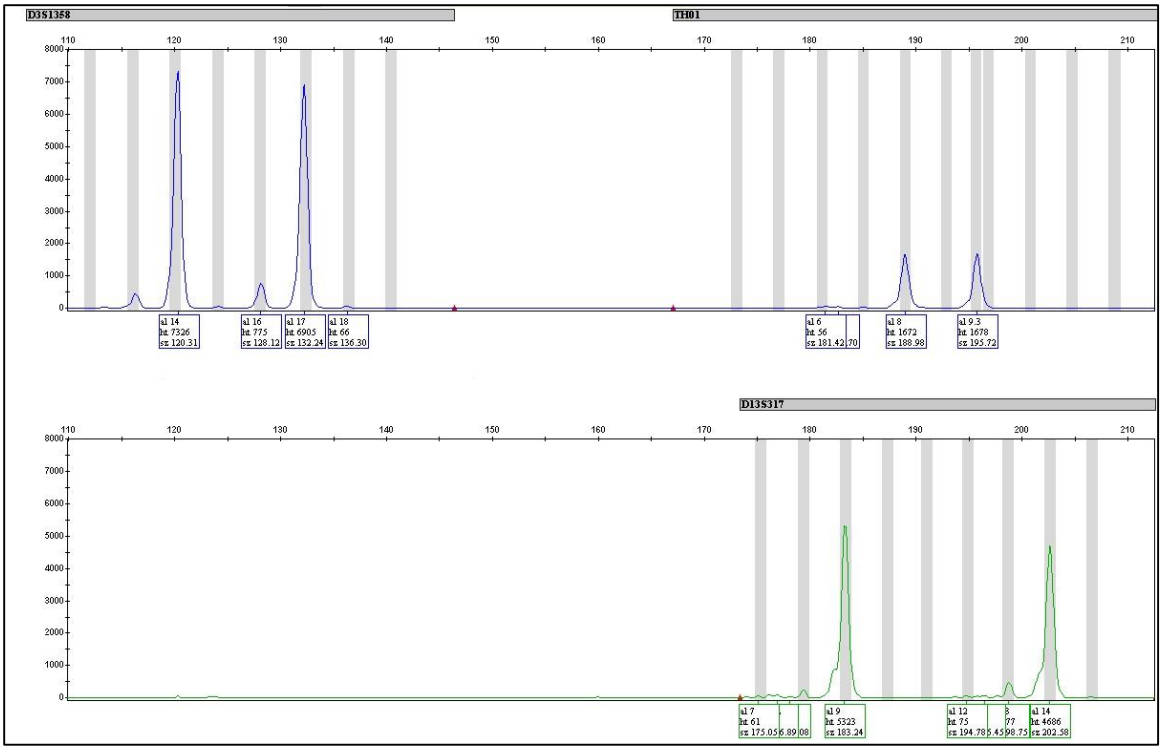


Figure 5. Electropherogram of sample dried with the nitrogen evaporator and collected with lysis buffer. The profile of the saliva donor was 14 and 17 at D3S1358, 8 and 9.3 at TH01, and 9 and 14 at D13S317.

When compared to the results from Chapter 2, a higher percentage of alleles were reported for the swabbed samples in this step. There are several possible reasons for the improved results. First, the current swabbed samples were amplified using Promega Go Taq® Hot Start Colorless Master Mix, compared to Promega PCR Master Mix in the previous Chapter. The use of a hot-start polymerase could have made the amplification more sensitive by reducing polymerase activity until 95°C and could have allowed for better replication of the DNA. The saliva used for these samples could have contained more DNA than the saliva used in Chapter 2, even though a smaller volume was used. Finally, the measured amount of distilled water used to moisten the swabs may have

allowed more DNA to stick to the swab, whereas in Chapter 2 no specific amount was used.

CHAPTER 4

# EVAPORATING WATER AND SALIVA IN RESEARCH SAMPLES

The previous chapters discussed the method development to concentrate and collect any epithelial cells from the liquid remaining in drink bottles. The samples used for research were dried with the nitrogen evaporator in a water bath and collected with ATL lysis buffer. For these samples, volunteers were asked to drink from water bottles leaving different volumes of water remaining as the variable being tested. Samples were collected from the bottoms of the bottles to determine the amounts of DNA at each volume of liquid. Samples were also collected from the tops of the bottles for the comparison of the experimental method to the established method.

## Methods & Materials

### *Sample Preparation*

Ten volunteers were asked to consume bottled water from three separate 16.9 fluid ounce (500 mL) bottles (Dasani, The Coca-Cola Company, Atlanta, GA), leaving a different amount of liquid in each bottle, 0 mL, 20 mL, and 40 mL remained. Each bottle was marked at the level for amount of water to be left in the container. The volunteers drank normally from each bottle and were advised to take between 20 and 30 sips from the bottles. All of the bottles were capped and stored under a hood in the lab until analyzed.

The remaining water was collected from the bottom of each bottle using the evaporation process described in Chapter 3. For bottles with 40 mL of liquid remaining, an additional swab moistened with 30 µL of distilled water was rubbed around the top of the bottle and inside the cap of the bottle. A buccal swab was also collected from each volunteer to be used a reference profile.

*Evaporating Samples*

Refer to Chapter 3 evaporating procedures of nitrogen evaporator and lysis buffer.

*Extraction*

Refer to Chapter 3 for extraction procedures.

*Quantification*

The DNA extracted from the bottom and top samples was quantified using an Applied Biosystems Quantifiler® Human DNA Quantification Kit (Foster City, CA) using the manufacturer's recommended protocol for a 25 µL reaction. Analysis was performed on a 7500 Real-Time PCR instrument (Applied Biosystems).

*Amplification and Analysis*

The extracted DNA was amplified using a customized three loci multiplex (D3S1358, TH01, and D13S317) and Promega Go Taq® Hot Start Colorless Master Mix (Madison, WI). The reaction was composed of 12.5 µL Promega Go Taq® Hot Start Colorless Master Mix, 6.5 µL of extracted DNA, and 5 pmol of each of the following primers:

D3S1358 forward primer – 5'-dye-ATGAAATCAACAGAGGCTTGC-3',

D3S1358 reverse primer – 5'-ACTGCAGTCCAATCTGGGT-3',

TH01 forward primer – 5'-dye-ATGAAATCAACAGAGGCTTGC-3',

TH01 reverse primer – 5'-ATTCAAAGGGTATCTGGGCTCTGG-3',

D13S317 forward primer – 5'-dye-GGCAGCCCAAAAAGACAGA-3',

D13S317 reverse primer – 5'-ATTACAGAAGTCTGGGATGTGGAGGA-3'.

Nuclease free water was added to bring the reaction to a total volume of 25 µL. DNA was amplified under the following conditions: denatured for 11 minutes at 95°C, held at 96°C for 1 minute, followed by 10 cycles of 94°C for 30s, 58°C for 30s, 70°C for 45s, then 22 cycles of 90°C for 30s, 58°C for 30s, 70°C for 45s, then held at 60°C for 30 min. The thermal cycler parameters were changed from those describe in previous chapters to increase the number of cycles from 30 to 32.

Amplified product was analyzed using Applied Biosystems 310 Genetic Analyzer and GeneMapper® ID Software v3.2 (Applied Biosystems). Amplified product was prepared by combining 1 µL of amplified product with 24.5 µL Fisher BioReagents™ formamide (Fisher Scientific, Pittsburg, PA) and 0.5 µL of GeneScan 500 LIZ Size Standard (Applied Biosystems). When performing analysis, peaks were recorded with a threshold exceeding 50 rfus.

Any amplification set with amplified alleles in the negative amplification control, or negative extraction control was re-amplified. The data from these amplification sets was not used. Any individual water bottle samples that contained alleles that did not originate from the volunteer were re-amplified. In these samples, alleles amplified from the donor were reported, and the additional alleles were noted.

## Results & Discussion

*Effect of Water Volume Remaining in Bottle*

The average quantities show the amount of DNA collected decreased proportionately with the water remaining in the bottle decreased (Table 1). The samples collected from the bottles with 40 mL of water remaining averaged 0.34 ng/µL, decreasing to 0.25 ng/µL for 20 mL samples and 0.04 ng/µL for 0 mL samples.

**Table 1.**
**Liquid Remaining in Bottles**

| **Volunteers** | 0 mL | 20 mL | 40 mL | Top |
|---|---|---|---|---|
| **A** | 0.00 | 0.37 | 0.04 | 0.23 |
| **B** | 0.00 | 0.08 | 0.06 | 0.17 |
| **C** | 0.07 | 0.00 | 0.22 | 0.10 |
| **D** | 0.03 | 0.05 | 0.33 | 0.35 |
| **E** | 0.00 | 0.21 | 1.21 | 1.17 |
| **F** | 0.11 | 0.24 | 0.28 | 0.32 |
| **G** | 0.03 | 0.20 | 0.38 | 0.44 |
| **H** | 0.00 | 0.18 | 0.37 | 0.21 |
| **I** | 0.00 | 1.13 | 0.48 | 0.28 |
| **J** | 0.18 | 0.00 | 0.00 | 0.12 |
| **Average** | 0.04 | 0.25 | 0.34 | 0.34 |
| **Std. Dev.** | 0.06 | 0.33 | 0.35 | 0.31 |

When evaluating the STR profiles, the same trend was observed for the 40 mL, 20 mL, and 0 mL samples. Combined, the 40 mL samples resulted in an average 4.2 of 6 alleles amplified (70%), with 5 of 10 samples resulting in full profiles. The 20 mL samples resulted in an average of 3.4 of 6 alleles amplified (57%), with 3 of 10 samples resulting in full profiles. The 0 mL samples resulted in an average of 1.8 of 6 alleles being amplified (30%), with only 1 of 10 samples resulting in a full profile.

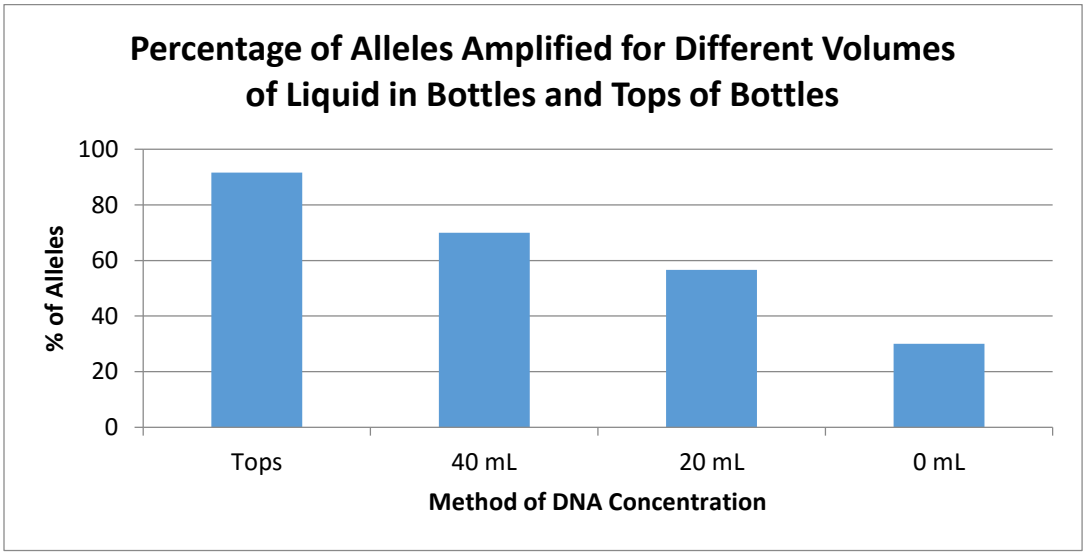


Figure 6. The graph depicts the percentages of alleles amplified using the three different volumes of liquid left in the bottles and the tops of bottles. For volume, samples with 40 mL of liquid (70%) resulted in more alleles being amplified than samples with 20 mL (57%) and 0 mL of liquid (30%). With comparison of 40 mL tops to 40 mL bottoms, the tops of bottles (92%) resulted in more alleles being amplified than the bottoms.

The 40 mL samples contained the highest average of DNA and resulted in the largest amount of alleles amplified. More DNA was present in the 40 mL and 20 mL samples than the 0 mL samples. Between the 40 mL and 20 mL samples, similarities were seen in the average amounts of DNA, but when comparing alleles amplified, the 40 mL samples resulted in a higher amount of alleles amplified. As more water is consumed the drinker starts to consume the DNA left in the bottle.

*Comparing Collection from Inside Bottle to Collection from Top*

Analysis performed on the DNA collected from the tops of the bottles showed a level of success similar to the analysis of the collections from bottles with 40 mL of water remaining. Using swabs to collect DNA from the tops of bottles submitted as evidence is the current procedure used in many labs. This comparison was performed to determine if

sampling from the bottom of the 40 mL bottles produced similar or better profiles than sampling the tops of bottles. The average quantity from samples collected from the tops of bottles (0.34 ng/µL) was similar to the 0.34 ng/µL average from the remaining 40 mL of water (Figure 5). The number of amplified alleles from bottle tops was higher (92%) than from 40 mL samples (70%) and the bottle tops resulted in 7 of 10 full profiles (Figure 6).

From the results, both the tops and the bottoms showed similarities in the average quantities of DNA, but when comparing the amounts of alleles amplified, the tops resulted in a higher count than the bottoms. Although the bottle tops had the highest number of alleles amplified, the results of the bottle bottoms showed that a significant amount of DNA can be found in the liquid which can result in partial or full profiles. While the Abaz [8] study also collected epithelial cells on the tops of water bottles, the DNA quantities in the Abaz study were lower than the averages of the top samples in this study. The Abaz study swabbed only the tops of bottles, which resulted in a smaller concentration of DNA. The combination of smaller surface and more exposure to environment could have affected any epithelial cells adhering to the tops of the bottles. A lab should consider targeting the bottoms of bottles because DNA may be present in the bottom when not found at the top. Saliva transfers to the bottle lid, and the lid may not be swabbed with the top of the bottle. The DNA on the bottle tops could be destroyed by accidental or intentional means, such as wiping off the top. The top of the bottle has more exposure to the environment than the bottom, and the environment could destroy any epithelial cells left on the top. Because the bottle bottoms had a significant amount of

DNA and can result in STR profiles, the bottle bottoms are a viable option to consider for analysis.

*Additional Alleles in Collected Samples*

During the analysis, additional alleles that did not originate from the volunteer were detected in several instances. For some amplification sets, alleles were amplified from extraction negative controls and amplification negative controls. When alleles were amplified in negative controls, the data from samples in that amplification set were not used. All samples were re-amplified after reagents were replaced and equipment was cleaned. In all instances, the additional alleles no longer appeared in the negative controls after re-amplification.

For other amplification sets, additional alleles that did not originate from the volunteer were detected in mock evidence samples. When additional alleles were detected in samples, the samples were re-amplified. On some occasions, the additional alleles were not re-amplified (Figure 7). In other instances, the additional alleles remained (Figure 8) or other additional alleles appeared (Figure 9). In all instances, the alleles amplified from the volunteer remained and were included in the reported data.

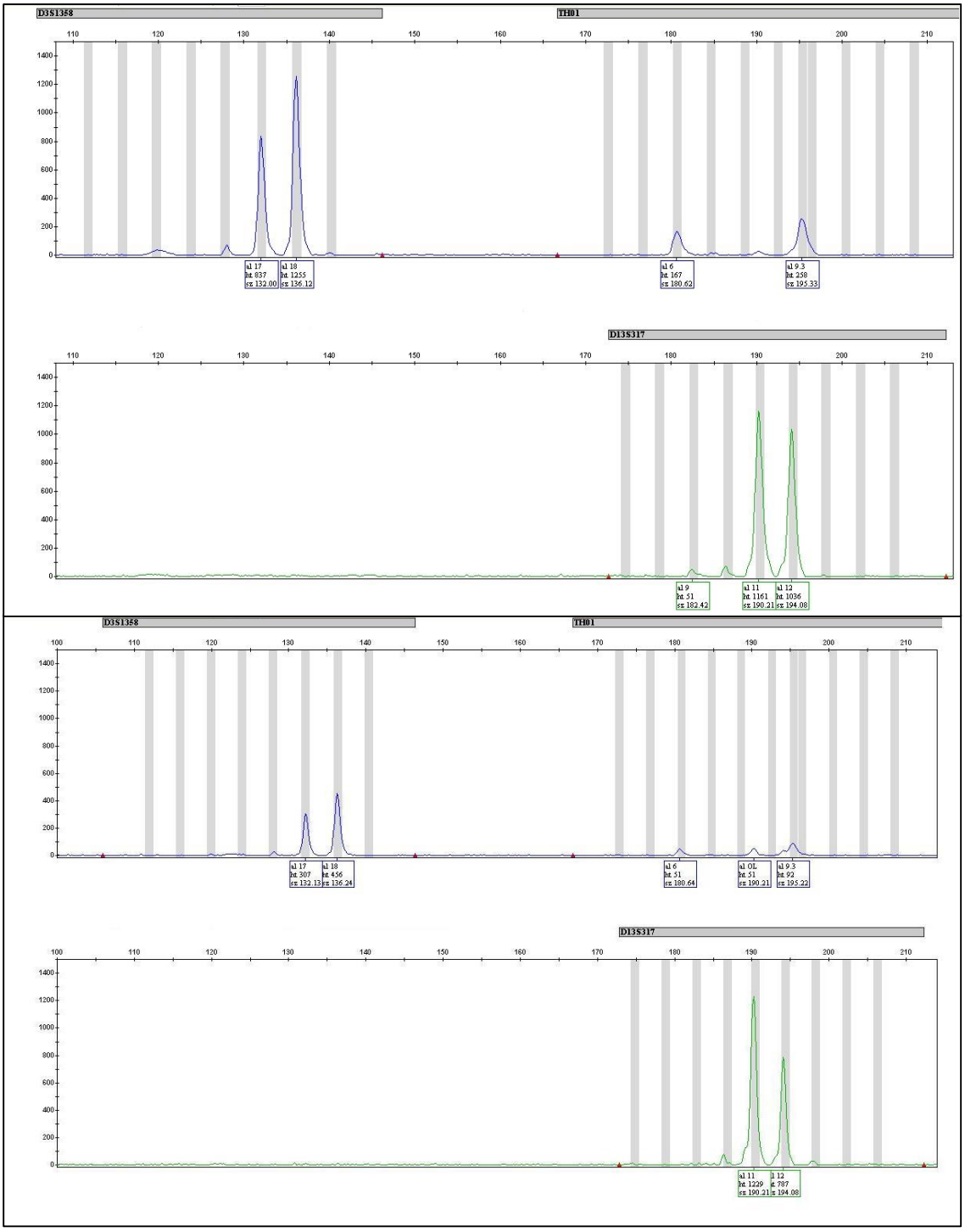


Figure 7. Electropherograms of sample with additional alleles not re-amplified. Each electropherogram represents an amplification of the same extraction. This extraction is from the sample with 40 mL left in a bottle consumed by Volunteer I.

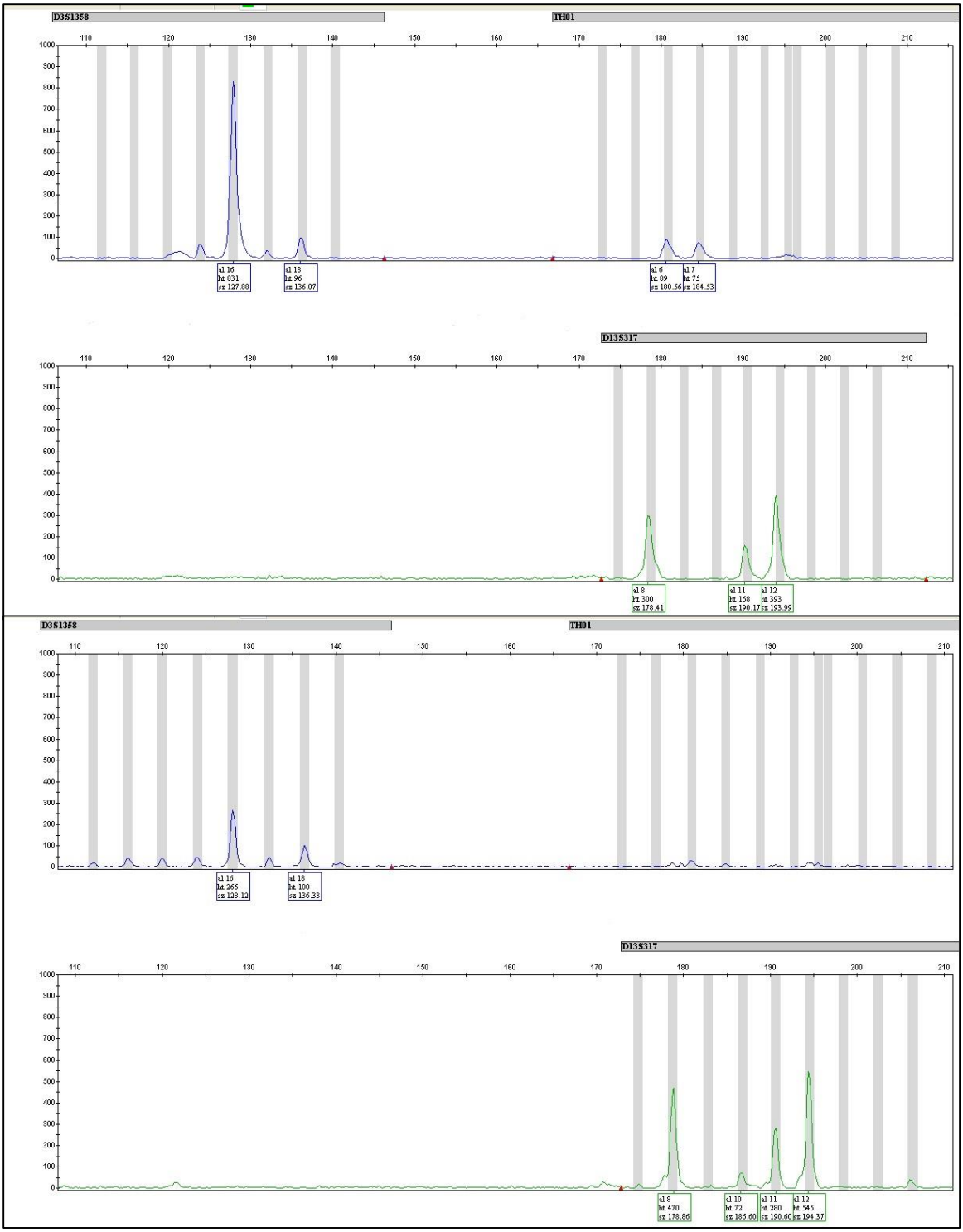


Figure 8. Electropherograms of sample with additional alleles re-amplified and remained the same. Each electropherogram represents an amplification of the same extraction. This extraction is from the sample with 40 mL left in a bottle consumed by Volunteer D.

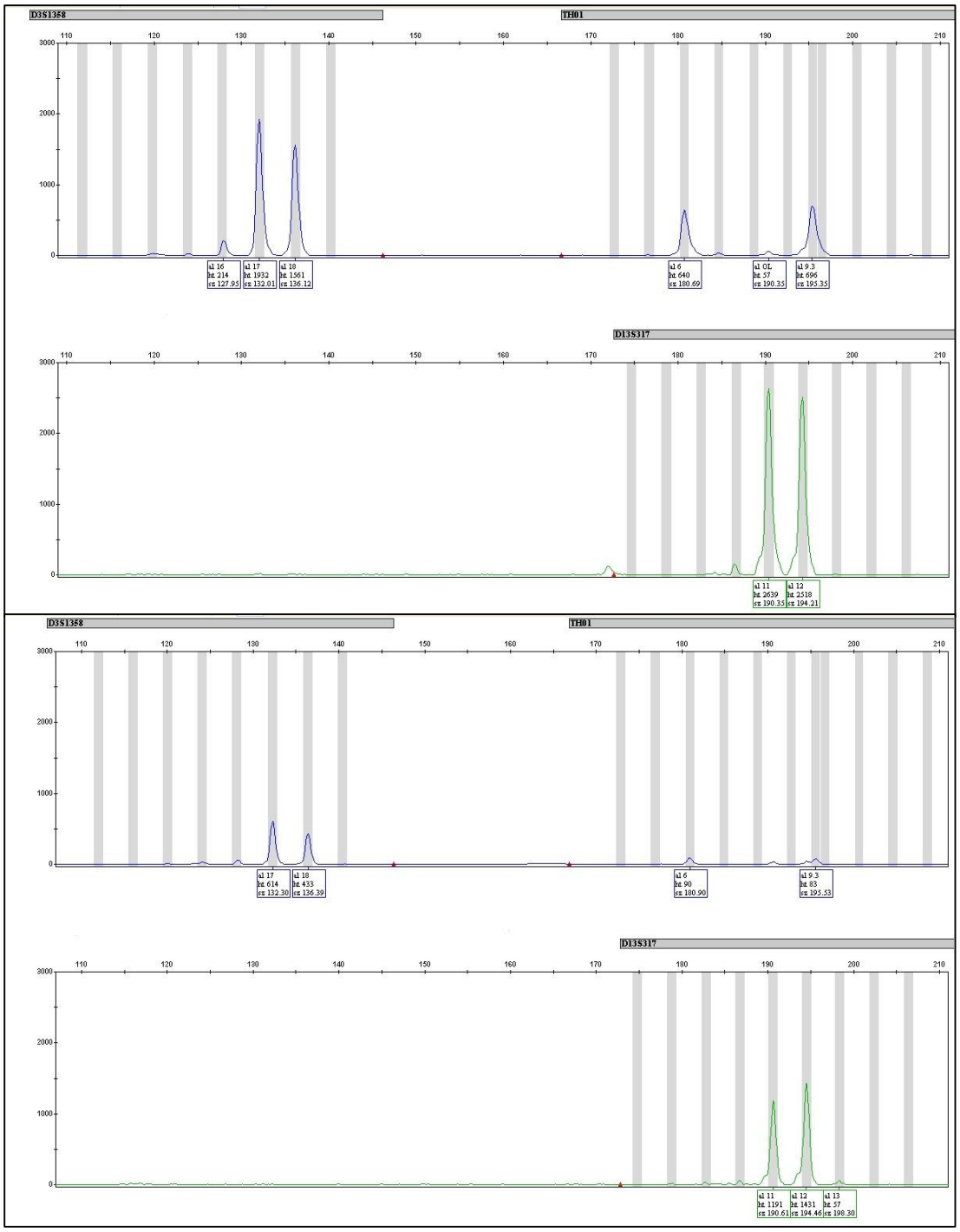


Figure 9. Electropherograms of sample with additional alleles re-amplified and differed from first analysis. Each electropherogram represents an amplification of the same extraction. This extraction is from the sample with 20 mL left in a bottle consumed by Volunteer I.

The additional alleles could have originated from several sources. One possibility would be during extraction. The cleanliness of the lab may not have been to the highest possible standards, and any cells on the table or in the air could have permeated the sample tubes. Additional alleles could have also appeared during amplification. Any lysed cells, previously amplified DNA, or previously extracted DNA could have seeped into one of the components needed for amplification. The water itself could have been contaminated by lax supervision during production or negligence of workers at the company. A likely source could have been from the volunteer. Any sharing of drinks or food prior to volunteering or kissing could have resulted in a mixed profile. Any of these possibilities could be the cause of the additional alleles.

CHAPTER 5

# CONCLUSIONS

The water bath with a nitrogen evaporator was the best of the three methods of DNA concentration tested in the first round of method development. The centrifuge had the lowest number of alleles called. The low count could have resulted from incomplete deposition of the epithelial cells into a pellet or from cells lysing in the supernatant. The evaporation in the oven yielded better results than the centrifuge, but the larger surface area of the bottles compared to the glass test tubes may have attributed to the lower results than the nitrogen evaporator. There is better access to the dried residue in the glass test tubes used for the nitrogen evaporator than in the bottle.

In the second round of method development, the combination of the water bath with the nitrogen evaporator and the lysis buffer was the best method for DNA concentration and collection. The nitrogen evaporator and the oven produced results similar in quality, but the nitrogen evaporator took less than half the time of the oven to dry samples, allowing for faster analysis of samples. For the collection, the lysis buffer and swab methods yielded similar results, but the lysis buffer eliminated the spin baskets from the extraction process.

The results show that more DNA can be collected from greater volumes of residue, within the tested range of 0-40 mL. The 40 mL and 20 mL bottom samples had more full and partial profiles than the 0 mL bottom samples, and higher peak height values than the 0 mL bottom samples. Quantitation also showed that as the volume of the

sample increased from 0 mL to 40 mL the amount of DNA left in the water also increased, and a majority of the 40 mL bottom samples had the greatest amounts of DNA. When comparing the 40 mL top samples to the 40 mL bottom samples, both the top and bottom samples had similar quality for the electropherograms and average quantities of DNA, but the top and bottom samples had differing numbers of alleles called and profile intensities.

Forensic biologists should consider targeting the bottoms of bottles submitted as evidence, as a significant amount of DNA could be present in the liquid. Saliva does transfer to the top of the bottle and the bottle lid, but the bottle lid may not be swabbed in addition to the top of the bottle. Unlike the bottom, the top of the bottle has more exposure to the environment, which could destroy any epithelial cells remaining on the top. Because this research has shown that a substantial amount of DNA is present in the bottoms of bottles and can result in STR profiles, targeting the bottle bottom should be an option for consideration during analysis.

*Future Directions*

Although collecting and analyzing the backwash remaining in bottles has not been considered before, the results of this research indicate that further experimentation should be done to fully evaluate the utility of this method. To further explore the research in this paper, an experiment using the Identifiler Kit would corroborate the usefulness of targeting bottle bottoms in a crime lab by possibly obtaining profiles with all 16 STR loci. Experiments with the type of drink as a variable would determine if the type of drink affects the recovery of DNA in the liquid, and varying the type of container may suggest that different containers affect the DNA left in the liquid differently. With the drinker as

the variable, the number of sips a person takes can cause the amount of DNA left in the liquid to increase or decrease. A certain number of sips would maximize the amount of DNA in the liquid whereas any higher or lower than that number of sips would decrease the amount of DNA left. Also, one drink could have multiple drinkers, and the liquid left in the bottle could contain different amounts of DNA left from the drinkers. When left outside, backwash in the bottle could be affected by environmental conditions. In addition to the evaluation done for this paper, more diverse experimentation, such as using the Identifiler Kit to amplify all 16 loci, the type of drink, type of container, number of sips influencing amount of saliva left, affect environmental evaporation on backwash, multiple drinkers from one bottle, or amount of time affecting backwash if bottle is left out to dry, would further the validity and significance of this area of DNA collection.

## References


[1] Caragine, Theresa; Mikulasovich, Rebecca; Tamariz, Jeannie; Bajda, Ewelina; Sebestyen, James; Baurn, Howard; and Prinz, Mechthild. Validation Testing and Interpretation Protocols for Low Template DNA Samples using AmpFISTR Identifiler. *Croatian Medical Journal.* 2009. 250-267.

[2] Alonso, Antonio; Anđelinoviæ, Šimun; Martín, Pablo; Sutloviæ, Davorka; Erceg, Ivana; Huffine, Edwin; Fernández de Simón, Lourdes; Albarrán, Cristina; Definis-Gojanoviæ, Marija; Fernández-Rodriguez, Amparo; García, Pilar; Drmiæ, Irena; Reziæ, Boja; Kuret, Sendi; Sancho, Manuel; and Primorac, Dragan. DNA Typing from Skeletal Remains: Evaluation of Multiplex and Megaplex STR Systems on DNA Isolated from Bone and Teeth Samples. *Croatian Medical Journal.* 2001. 42(3): 260-266.

[3] Kolowski, J.; Kolowski, J.; Petraco, N.; Petraco, N.; Wallace, M.; De Forest, P.; and Prinz, M. A Comparison Study of Hair Examination Methodologies. *Journal of Forensic Sciences.* 2004. 49(6).

[4] Harbison, S. A.; Petricevic, S. F.; and Vintiner, S. K. The Persistence of DNA Under Fingernails Following Submersion in Water. *International Congress Series.* 2003. 1239: 809-813, 2003.

[5] Webb, Laurance G.; Egan, Scott E.; and Turbett, Gavin R. Recovery of DNA for Forensic Analysis from Lip Cosmetics. *Journal of Forensic Sciences.* 2001. 46(6): 1474-1479.

[6] Sweet, D. and Hildebrand, D. Saliva from Cheese Bite Yields DNA Profile of Burglar: A Case Report. *International Journal of Legal Medicine.* 1999. 112: 201-203.

[7] Casey, L.; Engen, S.; and Frank, G. Quantitative Analysis of the DNA Distribution on Cigarette Butt Filter Paper. *Journal of Forensic Sciences.* 2013. 58(2): 470-473.

[8] Abaz, Jelena; Walsh, Simon J.; Curran, James M.; Moss, Delia S.; Cullen, Judi; Bright, Jo-Anne; Crowe, Gillian A.; Cockerton, Sarah L.; and Power, Timothy E.B. "Comparison of the Variables Affecting the Recovery of DNA from Common Drinking Containers," *Forensic Science International,* no. 126, pp. 233-240, 2002.

[9] Parys-Proszek, Agnieszka. Application of STR Analysis to the Profiling of Biological Traces Containing Low DNA Amounts. *Problems of Forensic Science.* 2004. 59: 100-114.

[10] Djuric, Marija; Varljen, Tatjana; Stanojevic, Aleksandar; and Stojkovic, Oliver. DNA Typing from Handled Items. *Forensic Science International: Genetics Supplement Series.* 2008. 1: 411-412.

[11] Daly, Dyan J.; Murphy, Charlotte; and McDermott, Sean D. The Transfer of Touch DNA from Hands to Glass, Fabric, and Wood. *Forensic Science International: Genetics.* 2011.

[12] Barbaro, A.; Cormaci, P.; and Barbaro, A. LCN DNA Typing from Touched Objects. *International Congree Series.* 2006. 1288: 553-555.

[13] Butler, J. M. Chapter 1: Sample Collection, Storage, and Characterization. *Advanced Topics in Forensic DNA Typing: Methodology*. Waltham, MA. Elsevier. 2012. 1-27.

[14] Hedman, Johannes; Dalin, Erik; Rasmusson, Birgitta; and Ansell, Ricky. Evaluation of Amylase Testing as a Tool for Saliva Screening of Crime Scene Trace Swabs. *Forensic Science International: Genetics.* 2011. 5: 194-198.

[15] Bartow, Mary K. *Personal Communications.* 8 February 2015.

[16] Johnson, Gretchen Zaneta. Concentration of Large Volume Biological Samples for Effective and Efficient Forensic DNA Analysis. Boston University School of Medicine. 2011.

[17] Shan, Zhi; Zhou, Zhongwu; Chen, Hui; Zhang, Zhiming; Zhou, Yi; Wen, Anxiang; Oakes, Ken D.; and Servos, Mark R. PCR-Ready Human DNA Extraction from Urine Samples Using Magnetic Nanoparticles. *Journal of Chromatography B.* 2011. 881-882: 63-68.

[18] Rogers, Nikki L.; Cole, Shelley A.; Lan, Hao-Chang; Crossa, Aldo; and Demerath, Ellen W. New Saliva DNA Collection Method Compared to Buccal Cell Collection Techniques for Epidemiological Studies. *National Institutes of Health American Journal of Human Biology.* 2007. 19(3): 319-326.

# APPENDIX

## IRB PROTOCOL FORM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

| | |
|---|---|
| Principal Investigator: | LINVILLE, JASON G |
| Co-Investigator(s): | KRIZ, BRITTANY |
| Protocol Number: | **X150702008** |
| Protocol Title: | *Detection And STR Profiling Of Saliva In Water Bottles* |

The IRB reviewed and approved the above named project on 8-25-15. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 8-25-15

Date IRB Approval Issued: 8-25-15

IRB Approval No Longer Valid On: 8-25-16

Member - Institutional Review Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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