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VALIDATION OF THE M-VAC® CELL COLLECTION SYSTEM FOR FORENSIC PURPOSES

by

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VALIDATION OF THE M-VAC® CELL COLLECTION SYSTEM FOR FORENSIC PURPOSES

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ABSTRACT

There is need for further development of cellular collection techniques in the field of forensic science. Currently, forensic analysts are limited to the use of swabs, taping, cutting, and scraping methods to collect cellular material. Each of these methods has its own benefits and drawbacks, however, none of them result in 100% recovery of the cells.

The Microbial Vacuum system (M-Vac®), developed by MSI, is a liquid based cellular collection system that was originally developed to collect microbes in the food-processing industry from various surfaces. This research represents a detailed study into the feasibility of utilizing the M-Vac® system for forensic purposes. Specifically, the phosphate buffer used with the M-Vac® was tested to confirm that it does not have a detrimental effect on cellular retrieval. Further, the ability of the M-Vac® to collect cellular material from a variety of substrates was tested. It was determined the M-Vac® can successfully collect both blood and semen from tile, denim, carpet, and brick materials in sufficient quantity for downstream PCR analysis.

Additionally, examination into whether DNA was dispersed during collection due to the significant force of impact of the liquid striking the surface was conducted. Specifically, areas surrounding the sample collection region were swabbed after collection with the M-Vac® and tested. Quantitative PCR analysis showed that DNA was retrieved up to 4 inches away from the collection area. This indicates that the M-Vac® system is a viable cell collection technique for forensic purposes, but only for samples which are isolated (i.e. where there is not another probative sample adjacent to it). If there are two probative samples within the same vicinity, then swabbing or taping is the recommended method of collection.

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ABBREVIATIONS

bp	Base Pair
DIH_2O	Deionized Water
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
µl	Microliter
M-Vac®	Microbial Vacuum
ml	Milliliter
ng	Nanogram
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SDS	Sodium Dodecyl Sulfate
STR	Short Tandem Repeat

1. Introduction

1.1 Background of DNA Analysis

In the past 30 years, the field of forensic science has undergone numerous developments in technology that allow scientists to detect and test more samples than ever before. Modern DNA analysis techniques allow a forensic DNA analyst to create a DNA profile unique to a specific individual. Originally, a DNA profile could only be obtained via the Restriction Fragment Length Polymorphism (RFLP) method, which needed a large amount of non-degraded DNA to be successful (1).

The RFLP method utilized the diverse lengths of DNA found between restriction enzyme digestion sites in order to create a profile unique to that individual. A restriction enzyme is a protein that cuts DNA when it recognizes a specific nucleotide sequence. The DNA sequences flanked by these sites vary in length between people, creating an individual profile for that person. For example, person A may have three EcoRI restriction sites, spaced 100 base pairs (bp) and 200 bp apart. When the DNA is digested, that individual will have a DNA profile that indicates the presence of RFLP lengths of 100, 200, and 300 bp (due to incomplete digestion). In contrast, person B may have only two restriction sites for EcoRI, spaced 500 bp apart. Therefore, that profile will exhibit only one, 500 bp RFLP length. The DNA fragments are then separated by gel-electrophoresis. Since DNA is negatively charged, a potential difference across the gel allows the smaller DNA fragments to travel farther in the gel matrix than the larger fragments, separating them based on size. When multiple restriction enzyme profiles are

combined, one can create a more individualized profile of that person's DNA, resulting in human identification (2-3).

Though a reliable method for DNA typing, the RFLP method is complicated and labor intensive. Also, RFLP analysis uses a large amount of DNA and many forensic samples contain low-levels of DNA. Therefore, RFLP is not an optimal technique for many biological samples obtained from crime scenes.

It was the development of the Polymerase Chain Reaction (PCR) by Kerry Mullis that allowed for the amplification of select DNA sequences, granting forensic scientists the ability to increase the detection capabilities of the DNA extracted from low-quality samples (4).

The PCR method allowed for a sensitive DNA profiling technique utilizing Short Tandem Repeats (STR). Like RFLP before it, the STR method relied on the variability in lengths of DNA sequences. Repeats are typically four or five base pairs in length and are repeated several times in succession. Instead of targeting restriction sites, a short primer (~20-30 bases) binds to the DNA surrounding the target STR. Once bound, the primer allows the enzyme polymerase to create a copy of the target sequence. When performed multiple times, the targeted sequence increases in concentration at an exponential rate. With the assistance of fluorescently labeled primers, the DNA copies created during the amplification process can be detected and quantified, allowing the scientist to determine the number of repeats (i.e. the alleles) (5-6).

One of the main benefits of the STR DNA typing method over the RFLP method is that the STR DNA fragments are separated using automated capillary electrophoresis.

Utilizing the same method of DNA separation as gel-electrophoresis, the matrix in CE is contained in a small capillary tube, reducing the amount of matrix and sample needed. After separation, the detector reads the fluorescent signal, originating from the fluorophore attached to the primer, as the DNA fragments pass the laser's window. The local southern method is then typically used to calculate the size of the fragment and comparison to an allelic ladder aid in the determination of which STR alleles are present (7-9).

Quantitative PCR (qPCR) is a real-time PCR method that determines the amount of DNA in a sample. The TaqMan® based quantitative PCR technique utilizes a probe during DNA amplification. This probe has a fluorescent tag attached to its end, and a quencher that masks the fluorescence of this tag when it is in close proximity to the fluorophore. During the amplification process, the DNA probe is degraded due to the endonuclease activity of Taq Polymerase, which releases the quencher, allowing fluorescence to be detected. The earlier in cycling the fluorescence is detected, the greater the amount of DNA present in the sample (10).

Quantification of DNA allows analysts to calculate the necessary volume of extract required for amplification, since too little sample will result in an incomplete profile. It also allows analysts to test the recovery of DNA from specific types of samples collected by different methods. Real-time PCR targets the amplification of specific DNA sequences, such as those specific to humans or male humans. This has made this method the preferred manner of DNA quantification in many forensic laboratories (9).

DNA profiling is given its power by modern studies in population genetics. These studies have determined the prevalence of certain alleles within the human population. By considering potential genotype combinations, calculation of the likelihood of a specific person having a specific genotype can be accomplished. An STR genotype is based on STR alleles present at a specific location. The combination of different alleles results in a person's genotype or 'type' (11).

When determining the commonality of a profile containing multiple genes, the individual gene frequencies can be multiplied together as long as Hardy-Weinberg equilibrium is maintained and the genes are independently inherited. The resulting statistic will show the likelihood of that genotype occurring at random in the population. When enough genes are taken into consideration, it is possible to create a genotypic profile that, based on the rules of statistics, is extremely rare. If enough loci are tested, the only time two people are expected to have identical profiles is in the case of identical twins (3).

Despite initial hesitancy to accept all types of DNA analysis - including low-level DNA analysis - in criminal trials, DNA analysis has now become a critical part of many investigations (12-13). In addition, DNA has also been used to exonerate the wrongly convicted and to solve cold cases that have remained unsolved for decades (14-15).

As DNA technologies became more sensitive, modern forensic chemical tests for detecting biological samples have also become increasingly sensitive. For example, the chemical luminol, which emits blue fluorescence when exposed to the peroxidase-like activity of hemoglobin and, can detect the presence of hemoglobin up to a 1:5,000,000

dilution. Leucomalachite green (LGM) and the Kastle-Meyer (KM) presumptive blood tests detect hemoglobin at dilutions of 1:100 and 1:100,000, respectively, under the same conditions (16). While any stain that shows a positive signal when tested with a presumptive test should be collected, it is possible that there is simply not enough cellular material in one localized area to yield a DNA profile. One would be required to collect from a larger area in order to collect as many cells as possible, but that is not feasible since many biological stains are collected using sterile swabs.

The efficiency of a swab to collect biological material is limited by its surface area and absorbency. There are also issues associated with the retrieval of the sample from the swabbing material during DNA extraction. Additionally, not all swabs are made from the same material and will yield different amounts of sample. For example, a study conducted by the NSFTC tested cotton, rayon, polyester, foam, nylon, etc., to determine which yielded the most DNA. Utilizing a single swab, the study collected 2 µl of dried blood from a glass slide and extracted the DNA. Pur-Wraps® polyester-tipped applicator, Copan Nylon® flocked swabs, and Forensic ID – Trigger ID^{TM} all yielded approximately 46% of the DNA available. The Puritan® - Self-Saturating Swab, a Trace DNA collection device, yielded only approximately 11% and a single cotton swab yielded 25.2% (17).

Additionally, this study also compared the amount of DNA retrieved when blood was collected using a single swab to when the double swab technique was utilized. As previously mentioned, a sterile cotton swab yielded approximately 25.2% of the DNA present in the original 2 µl stain collected. This yield doubled to 53.5% when the double

swab technique was utilized. A single foam swab yielded approximately 36.4%, with the double swabbing increasing the yield to approximately 61.1% (17).

As previously mentioned, the double swab method has been suggested to be a superior method of collection of cellular material. Originally developed for collecting epithelial cells in saliva from bite marks - where rubbing too hard with the collection swab can result in more victim DNA being collected than cells of interest - this method first collects the sample using a wet swab and then follows up with a dry one and has become the preferred method of collecting biological samples (18).

Taping is another method commonly used at crime scenes or in forensic laboratories to collect trace or biological evidence, especially from larger surface areas. Taping involves repeatedly placing the adhesive side of a piece of tape on the surface that contains the biological material. Hairs, fibers, and cellular material are collected onto the tape, from which they can later be recovered for analysis. Recent publications suggest the taping method may be better than swabbing for collection of epithelial cells from objects that have been touched. For example, Barash *et al.* successfully characterized DNA profiles from cells collected via taping from high friction areas on the inside of a ski mask, the sides, trigger, hammer and cylinder of a revolver, and interior of a woolen glove. Additionally, they were able to successfully collect DNA from the interior and exterior of seed shells via taping - a substrate they had been unable to effectively collect from with swabbing (19).

It is possible to extract DNA from a biological fluid without first having to collect it onto a swab. This can be accomplished by cutting a small section of the substrate that

is expected to contain a biological fluid/stain. This eliminates possible loss of sample to the swab material. Petricevic *et al.* extracted DNA from 3 cm^2 cuttings from lower bed sheets. The first phase of the study had volunteers sleep on the new sheet in their own beds and their other regular bedding. Cuttings were taken from the upper shoulder, mid body, and foot area of the bedding afterwards. DNA collected ranged from 1-8 ng. The second phase had the volunteers sleep with a new lower sheet in foreign beds with that bed's regular bedding. Cuttings from the same areas as the previous phase were collected. The DNA collected was most often predominantly from the volunteer sleeper, with only trace amounts of the foreign bed's normal occupant detected. They concluded that even after one night in a bed, DNA evidence can be obtained from shed epithelial cells of an individual (20).

When needing to collect cellular material or trace evidence from a large evidence item, such as a bed sheet, forensic scientists can utilize the method commonly known as scraping. This method involves scraping the material with a spatula or blade and collecting the loosened material for downstream analysis. The main problem associated with this method is determining the method by which to recover the biological material once it has been scraped from the surface. One study collected scrapings into a pill box and then swabbed the pillbox with a moist swab to collect the desired cells. Despite the two-step process involved during scraping, the scraping method resulted in more DNA material being collected than swabbing the evidence alone (21).

Despite the studies aimed at characterizing DNA recovery and the advances in STR typing methods, more innovation is needed with regard to how biological samples

are collected (22). It is important that research in this area continues since successful DNA typing is highly dependent on the number of cells collected.

Once a biological sample has been collected, a forensic scientist has a few options concerning how to go about extracting the DNA material depending on the sample type. The phenol-chloroform method has long been used in forensic labs to extract DNA from highly decomposed samples, bone, and sexual assault samples that contain epithelial and sperm cells. However, this method utilizes the toxic chemical phenol and must be performed in a fume hood. Although this method recovers significant amounts of DNA, it also produces a sample that contains RNA and proteins, which will need to be removed from the sample in a follow up procedure. This process also involves multiple transfers of the sample into different tubes, which increases the chance of contamination (23).

Chelex® is an ion-exchange method of DNA extraction. This method is less time-intensive than traditional organic extraction and utilizes fewer tube transfers. However, Chelex® extraction is not ideal for samples that may be stored for long periods of time before analysis. Greenspoon *et al*. showed that multiple freeze-thaw cycles of Chelex® DNA samples resulted in increased levels of allelic drop out which reached up to ~ 30%. This level of drop out was not observed in samples that had not been frozen before analysis. In comparison, samples isolated via the QIAamp® DNA extraction method rarely showed allelic drop-out or signal loss (24).

The QIAamp® DNA extraction technique utilizes a column with a silica-based membrane to purify DNA. There are a number of advantages associated with the QIAamp® method of extraction. The buffers that the columns are washed with remove

contaminants such as RNA and proteins from the sample without removing the DNA from the silica membrane, resulting in a purified sample. Low amounts of DNA can also be recovered from low-level samples with this method (25).

1.2 Microbial Vacuum Cell Collection System

The Microbial Vacuum Cell Collection System (M-Vac®, MSI, Buffdale, UT) is a liquid rinse vacuum collection system originally developed for the food processing industry to collect microbes from the surface of large cuts of meat. The M-Vac® works by first spraying a buffer solution onto a surface via a nozzle while simultaneously suctioning that buffer to a sterile bottle. This buffer is expected to contain cellular material that may have been present on the surface. This method of wet-vacuum collection could be an alternative method of evidence collection for very diffuse or dilute biological samples.

The main drawback of using a wet-vacuum system is the fact that in collecting the biological material, the cells are suspended in a large volume of buffer. The DNA extraction techniques utilized in a forensic laboratory setting are not equipped for processing a sample that is hundreds of milliliters in volume.

However, previous studies by Johnson suggest that concentration of large volume samples can be accomplished by collecting the cellular material onto a membrane filter, which would then go through the DNA extraction process. Interestingly, this study also suggested that care must be taken when choosing a large volume collection buffer as buffers containing surfactants such as SDS were shown to have a negative effect on DNA yields (26).

To test the applicability of the wet-vacuum collection method for forensic purposes, Sorenson Forensics (Sorenson Forensics Inc, Salt Lake City, UT) completed an initial study which characterized the recovery rates of vacuum collection, swabbing, and cutting methods. They compared the DNA recovered from wet-vacuum collection samples to the DNA recovered from cotton swabs and small fabric cuttings of saliva and blood on various substrates. All experimental samples collected were concentrated using Amicon® concentration filters (Millipore Corperation, Billericia, MA) before extraction (27).

In part one of the study, 500 µl of a dry 1:2 dilution of a saliva stain were collected using either one wet swab, one dry swab, or the wet-vacuum method. The two wet-vacuum samples collected had an average concentration of 1.13 ng/µl of DNA. Only one swab sample had recoverable DNA, with a concentration of 0.02 ng/µl (27).

In part two, 500 µl of a 1:10 dilution of blood were spotted onto white cotton, nylon, polyester, and denim. Wet-vacuum samples and 1 cm^2 cuttings were collected. When quantified after robotic set up, the wet-vacuum collected samples from cotton, denim, and polyester were determined to contain a higher concentration of DNA than the samples collected via cutting from the same substrates. Interestingly, when quantification was set up manually, the cuttings from denim yielded less concentrated DNA than the wet-vacuum sample. Wet-vacuum samples collected from nylon consistently contained less DNA then the cut samples. In response to the results from nylon, an additional bloodstain was collected via wet-vacuum and swabbing from the

nylon substrate. The wet-vacuum sample resulted in a more concentrated sample than the swab sample (27).

Part three of the study consisted of neat saliva spotted onto nylon, polyester, and denim. Wet-vacuum samples and wet and dry swabs were collected. Quantified after robotic set up, the wet-vacuum collected samples from nylon, polyester, and denim were determined to contain a higher concentration then the samples collected via cutting from the same substrates (27).

As a result, the Sorenson study concluded that wet-vacuum collection was able to collect a sufficient level of DNA from all substrates. Successful amplification of collected samples by the Identifiler^{TM} multiplex STR kit (Applied Biosystems, Foster City, CA) was also reported (27).

Further, the Garrett study compared the wet-vacuum collection to the double swab and taping collection methods on tile, carpet and denim substrates. Volumes of 75, 7.5, 0.75, and 0.075 µl of blood were tested. Vacuum collection performed comparably and sometimes better than one or both of the other methods when collecting from tile. On denim, the vacuum technique consistently collected more DNA than the other two methods. On carpet, the vacuum method collected equal or greater amounts of DNA than recovered by swabbing and taping. As a result, Garrett *et al.* recommended using the wet-vacuum collection technique on limited types of samples, particularly when the location of the stain is unknown or the biological material is not visible and expected to range over a large surface area (28).

1.3 Purpose of Study

The purpose of this study was to determine important aspects for consideration when working with a wet-vacuum cell collection system from a forensic viewpoint.

Because many forensic laboratories rely upon state and federal funding, it is important to ensure cost efficiency when processing DNA samples. The commercially available M-Vac® handsets are disposable, but it was of interest to test the possibility of reusing handsets during either validation or training. Therefore, a study designed to examine and develop a method to clean the handsets to allow for repeated use without the risk of contamination was conducted.

Forensic DNA analysis has a low limit of detection; previous studies suggest the LOD (limit of detection) is in the nanoliters range (29). Given the low LOD and complications associated with interpretation of low-level DNA typing and drop-in, it was of interest to determine if the force of the spray used during vacuum collection would result in a sample being spread to the surrounding area, thus increasing the possibility of cross-contaminating other samples in range (30).

Finally, this study examined the efficiency of the M-Vac® in collecting a variety of volumes (100 µl, 10 µl, 1 µl, and 0.1 µl) of blood and semen samples from diverse surfaces (brick, denim, carpet, and tile) that may be encountered in forensic casework.

2. Materials and Methods

All materials were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. All aspects of this study were conducted in compliance with the

ethical standards set by the Institutional Review Board of Boston University School of Medicine.

2.1 General Methods

2.1.1 QIAGEN Extraction of Blood and Semen Samples

All samples were extracted using the QIAamp® DNA Investigator Kit, using the manufacturer's recommended protocols (25).

2.1.1.1 Neat Blood Samples

The *Isolation of Total DNA from Small Volumes of Blood or Saliva* was performed according to the manufacturer's recommended protocols for samples of neat blood (0.1-100 µl) (25). Buffer AL contained 1 µl of carrier RNA for every 100 µl of lysis buffer as per the manufacturer's recommendations.

A general description of the protocol used was as follows: A volume of 100 µl neat blood, 10 µl of blood plus 90 µl DI H_20, and 1 µl of blood plus 99 µl DI H_2O were placed in a clean 1.5 ml microcentrifuge tube. The most dilute sample was created by using 10 µl of 1:99 dilution and mixing it with an additional 90 µl of DI H_2O to achieve the desired 1:999 dilution. Buffer ATL, Proteinase K, and buffer AL were added at their recommended volumes. Samples were then incubated at 56ºC for 10 minutes. After incubation, 50 µl of 200 proof ethanol was added to the tubes and the samples were then transferred to the QIAamp® MinElute column. The column was washed with 500 µl buffer AW1, 700 µl buffer AW2, and 700 µl 200 proof ethanol. After centrifugation, 25 µl of buffer ATE was applied to the center of the QIAamp® MinElute column's

membrane. Because the final eluent was up to 5 µl less than the volume of the elution buffer added, the final volume retrieved was assumed to be 20 µl.

2.1.1.2 Neat Semen Samples

The relevant parts of *Isolation of Total DNA from Sexual Assault Specimens* were performed according to the manufacturer's recommended protocols for samples of neat semen (0.1-100 µl) (25). Buffer AL contained 1 µl of carrier RNA for every 300 µl of lysis buffer.

A general description of the protocol used was as follows: A volume of 100 µl neat semen, 10 µl of semen plus 90 µl DI H_2O, and 1 µl of semen plus 99 µl DI H_2O were placed in clean 1.5ml microcentrifuge tubes. The most dilute sample was created by using 10 µl of the 1:99 dilution and mixing it with an additional 90 µl of DI H_20 to create the desired 1:999 dilution. A volume of 280 µl Buffer ATL, 15 µl Proteinase K, and 15 µl 1M DTT were added. The samples were then incubated at 56ºC for 1 hour and after incubation, 300 µl of buffer AL was added. The samples were then incubated at 70ºC for 10 minutes. After a 1-minute spin at maximum angular velocity, 150 µl of 200-proof ethanol was added. The samples were then transferred to the QIAamp® MinElute column and the column was washed with 500 µl buffer AW1, 700 µl buffer AW2, and 700 µl 200 proof ethanol. After centrifugation, samples were incubated at room temperature for 10 minutes with their tubes open. Following this, 25 µl of buffer ATE was applied to the center of the QIAamp® MinElute column's membrane. The sample was then incubated for another 5 minutes at room temperature. Because the final eluent

was up to 5 µl less than the volume of the elution buffer added, the final volume retrieved after the final was assumed to be 20 µl.

2.1.1.3 Filtered Blood and Semen Samples

Large volume samples containing blood or semen that were filtered using the filtration-concentration method were extracted in the same manner as the neat blood or semen samples but with a few additional steps.

The steps were as follows: The round Millipore filters were cut with clean scissors to ~0.5 cm squares. These squares were then separated into two 1.5 ml microcentrifuge tubes. The tubes were treated as separate samples and combined later in the extraction process. A piggyback spin using a plastic mesh filter (EMD Millipore, Billerica, MA) was added before the addition of the 200 µl of ethanol to remove all lysate from the filter material. This lysate was then added to the QIAamp® MinElute Columns as before, with the addition of an extra centrifugation so that all lysate could pass through the membrane.

2.1.1.4 Swab samples

The *Isolation of Total DNA from Surface and Buccal Swabs* was performed according to the manufacturer's recommended protocols for biological samples collected on a cotton swab (25). Buffer AL contained 1 µl of carrier RNA for every 400 µl of the lysis buffer.

A general description of the protocol used was as follows: The cotton of the swab was removed from the wooden stick using a sterile scalpel and then placed in a 1.5 ml microcentrifuge tube. A volume of 20 µl Proteinase K and 400 µl buffer ATL were

added and the samples were incubated at 56ºC for 1 hour. After incubation 400 µl of buffer AL was added. The samples were incubated at 70ºC for 10 minutes after which 100 µl of 200-proof ethanol was added to the tubes. A piggyback spin was performed to remove any lysate from the cotton swab after which the samples were then transferred to the QIAamp® MinElute column. The column was then washed with 500 µl buffer AW1, 700 µl buffer AW2, and 700 µl 200 proof ethanol. After centrifugation, samples were incubated at room temperature for 10 minutes with their tubes open. Following this, 25 µl of buffer ATE was applied to the center of the QIAamp® MinElute column's membrane. The sample was then incubated for another 5 minutes at room temperature. Because the final elutant was up to 5 µl less than the volume of the elution buffer added, the final volume retrieved after the final was assumed to be 20 µl.

2.1.2 DNA Quantification

Samples were quantified using the Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA) and the ABI 7500 Sequence Detector System (Applied Biosystem, Foster City, CA). Amplifications were performed with a final reaction volume of 25 µl following the manufacturer's recommendation (31). One standard curve was utilized for all concentration calculations (32).

2.1.3 Filtration-Concentration Method of Large Volumes

Vacuum collection resulted in a collection volume up to 150 ml, so the biological material first had to be concentrated or filtered from the larger volume before the DNA could be extracted using approved methods. This filtration process utilized in this work used the Durapore® membrane. This membrane consists of polyvinylidene fluoride and

was 47 mm in diameter and contained 0.45 µm pores. To concentrate cells, the large volume of liquid was filtered through the membrane, whereby cells were expected to remain on the surface of the filter. All glassware was cleaned using a 10% bleach solution followed by a 70% ethanol solution.

Specifically, the filtration-concentration process consisted of the following steps: A reusable metal filter was placed securely into the base of the glass filter holder and covered with one Durapore® membrane filter. The top of the glass funnel was then connected. With the vacuum pressure on, the large volume sample was added to the funnel and allowed to filter through. The interior of the funnel was rinsed with DI H_2O. The Durapore® membrane filter was removed with clean tweezers and placed in a clean weigh boat. The filter was then cut for downstream DNA processing.

2.1.4 Statistical Data Analysis

Microsoft Excel Data Analysis Tool Pak (Microsoft, Redmond, WA, 2010) was utilized for descriptive and graphical analysis. All error bars reported are two standard deviations from the mean. Percent DNA recovery was determined for every volume of blood and semen by dividing the DNA yield observed via vacuum collection by the DNA yield of the neat biological sample.

2.1.4.1 Propagation of Error

The error associated with percent recovery was calculated using the theory of propagation of error, where A represents the mean amount of DNA recovered using the M-Vac® collection system, σ_A represents the error associated with A, B represents the mean amount of DNA recovered from a neat biological sample, σ_B represents the error

associated with *B*, *%Recovery* is the average percent of DNA recovered via the M-Vac® collection system, and $\sigma_{\%Recovery}$ is the standard error associated with *%Recovery*.

$$\frac{\sigma_{\%\,Recovery}}{\%Recovery} = \sqrt{(\frac{\sigma_A}{A})^2 + (\frac{\sigma_B}{B})^2} \qquad \text{(Equation 1)}$$

2.2 Sample preparation

2.2.1 Large Volume Samples

To determine the yield of DNA from large volume samples following the vacuum filtration method, as well as to see if exposure to the phosphate buffer had any negative effects on DNA yield, a sub-set of samples were filtered from the buffer without having been collected using the Microbial Vacuum. Volumes of 100 µl and 50 µl neat blood were added to 150 ml of phosphate buffer. This was performed in quadruplicate so that an average recovery could be determined. Additionally, for comparison purposes, the same concentrations of blood were added to 150 ml of DIH_2O, again in quadruplicate, and the mean percent recovery was calculated.

2.2.2 Samples for Collection by the M-Vac®

Four substrates were chosen to examine the efficiency of vacuum collection for forensic purposes. These substrates were tile, brick, carpet, and denim. All substrate items were previously unused. The tile was first cleaned by soaking in a 10% bleach solution for 10 minutes and then wiped with 70% ethanol. The brick was cleaned using water to remove any loose cement fragments and dirt. The carpet and denim were used without additional cleaning. Four volumes were chosen to test the recovery of the vacuum collection. The volumes were 100, 10, 1, and 0.1 µl. These volumes were tested

using both neat blood and neat semen samples. Volumes were spotted onto the chosen substrate via pipette and allowed to fully dry before collection.

2.3 Sample Collection

2.3.1 Collection Using the M-Vac®

Before using the Microbial Vacuum, the collection bottle was screwed on securely and all tubing was set up according to manufacturer's guidelines. The handset was placed flush against the substrate. The vacuum suction and then the buffer spray were turned on. The handset was moved in a circular motion with light pressure on the substrate until the collection volume reached 150 ml.

2.3.2 Contamination Study

The Microbial Vacuum Cell Collection System collects cellular material by spraying a buffer from the collection handset while simultaneously vacuuming it up. Because the buffer is sprayed onto the surface, there is a chance that the sample could be propelled by buffer onto the surrounding area, thus risking contamination of surrounding samples. To test this, tiles containing 100, 10, 1, or 0.1 µl of blood were surrounded on all sides by four clean tiles. The blood sample spotted on the center tile (Figure 1) was then collected using the wet-vacuum technique.

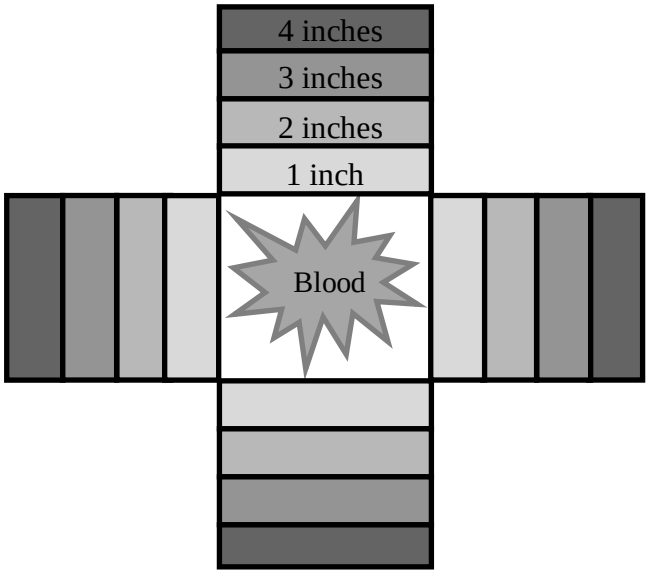

Figure 1: Schematic of the clean tiles arranged around the sample tile containing 100, 10, 1, or 0.1 µl. The shaded areas represent the 1, 2, 3, and 4 inch sample areas that were collected via swab for DNA.

Following collection, the surrounding tiles were swabbed using the double swab technique at 1-inch increments to collect any sample that may have spread during the wet-vacuum collection process (Figure 1). This was performed in quadruplicate for each sample volume, so that there were four swab pairs for each distance from the central sample per volume. These swabs were extracted using the *Isolation of Total DNA from Surface and Buccal Swabs* protocol and quantified for total DNA.

2.4 Cleaning Methods

2.4.1 Cleaning the M-Vac® Handsets

The Microbial Vacuum Cell Collection System consists of a vacuum and a replaceable collection handset. A full description of the system has been given previously (26). The handsets consist of the central tubing in which the sample is collected, a smaller tube which transports the buffer to the nozzle, the collection bottle area, and the plastic headset casing. While the handsets are disposable, it of interest to

determine whether these handsets could be reused and whether a cleaning method could be developed to enable the reuse of the handsets. Thus, three different cleaning methods were developed and tested.

These methods all utilized bleach and ethanol as cleaning reagents. These were chosen since they are commonly employed cleaning reagents in forensic laboratories. Exposure to bleach will cause degradation of DNA and formation of thymine dimers, which prohibit PCR amplification (33), and ethanol is used to remove any remaining cellular material and bleach from the surface.

2.4.1.1 Cleaning Methods 1 and 2

A new, unused handset was used to collect a dried sample of 100 µl of blood from a tile. This handset was then rinsed with 100 ml DIH_2O. It was then submerged in a bath of 10% bleach for 15 minutes for Method 1 and 20 minutes for Method 2. Particular attention was paid to the interior tubing of the handset, to ensure that it was completely filled with the bleach solution. After the soak, the tubing was rinsed with 100 ml of 70% ethanol, followed by another rinse with 100 ml DIH_2O.

This 'cleaned' handset was then used to collect a blank sample from a clean tile. The collected sample was filtered, extracted, and quantified as previously described to determine whether or not DNA was observed in the sample. Each cleaning method was completed 12 times or until a positive signal was observed. If a DNA signal in a blank was observed, the cleaning method was determined unsuitable for forensic purposes due to potential sample-to-sample contamination.

2.4.1.2 Cleaning Method 3

Method 3 differed from Methods 1 and 2 in that it did not use a timed exposure to the 10% bleach solution. Instead, the handset was rinsed with 100 ml DIH_2O. This was followed by flushing the tubing with 500 ml 10% bleach and 250 ml 70% ethanol. Finally, the handset and tubing was rinsed with 100 ml DIH_2O. The final rinse was incorporated to ensure any remaining ethanol was removed from the system (34).

2.4.2 Cleaning of the M-Vac® Collection Bottles

The Microbial Vacuum Cell Collection System collected the buffer and biological sample into a plastic collection bottle. To minimize cost of using the Microbial Vacuum, these bottles were cleaned for reuse. The cleaning method used consisted of a rinse with DIH_2O that was followed by a 10-minute soak in a 10% bleach solution. The bottles were then inverted onto the lid and soaked for an additional 10 minutes. The bottles were rinsed with 70% ethanol, followed by DIH_2O, and allowed to air dry.

3. Results and Discussion

3.1 Cleaning the M-Vac® Handsets

Three cleaning methods were developed and tested to determine whether the collection handsets could be cleaned and reused. Methods 1 and 2 were inefficient methods of cleaning the handsets. Of four blank samples tested for Method 1, two resulted in positive DNA results, with an average DNA concentration of 0.001 ± 0.002 ng/µl of sample. Of the twelve blank samples tested using Method 2, four resulted in positive DNA results, with an average DNA contamination of 0.005 ± 0.006 ng/µl of sample. Given the low limits of detection associated with forensic DNA processing and

the need to ensure minimal levels of sample-to-sample carryover, Methods 1 and 2 were determined to be insufficient for forensic purposes.

In contrast, Method 3 resulted in twelve blank samples which showed no indication of DNA during qPCR analysis. The twelve blanks were collected in groups of four over three different days to ensure reproducibility of null results over extended time periods. This indicates that this cleaning method was reliable and was therefore used throughout the remainder of the study. The agitation provided by the vacuum pressure is believed to play a key role in the success of this method. Not only does it expose the interior of the tubing to a constant flow of the cleaning solutions, it is hypothesized that the agitation also aids in removing debris that may have adhered to the tube walls.

3.2 Confirmation of Percent Yield Following Vacuum Filtration and Extraction

The filtration method used to concentrate the large volume samples produced by vacuum collection has previously been described. The aforementioned study showed that the average percent recovery of DNA after large-volume extractions was approximately 50% when samples were filtered from DIH_2O. It was also determined that the type of buffer from which the sample is being filtered will affect the overall recovery (26).

As a result, preliminary studies to test the feasibility of utilizing phosphate buffer – i.e. the buffer provided with the system – as the collection buffer were performed. Volumes of 100 µl and 50 µl of blood were filtered from 150 ml of phosphate buffer (pH 7) and compared to the results of the same volumes when filtered from DIH_2O. The average percent yield for samples from the phosphate buffer were 53 ± 21% and 81 ±

23% for 100 µl and 50 µl, respectively. Average percent yields for samples from water were 40 ± 32% and 116 ± 70% for the same volumes.

The excess in yield for the smaller 50 µl volume from water is attributed to stochastic processes and uncertainty in analytical measurement associated with DNA analysis (35-36). Even so, the data show that the phosphate buffer does not have a detrimental effect on cellular concentration and corroborate the average yield of approximately 50% previously published.

3.3 Contamination Risk of Using a Wet-vacuum Collection System

The wet-vacuum collection system sprays the phosphate collection buffer from the center of the handset with substantial force. Therefore, it was of interest to determine whether this force is significant enough to propel cellular material from the area of interest to the surrounding area, thus risking contamination of samples in the vicinity.

Table 1: Concentration of DNA detected via qPCR after swabbing the tiles surrounding the area of interest. ND = not detected.

Volume of blood	DNA(ng/µl) at 1”	DNA(ng/µl) at 2”	DNA(ng/µl) at 3”	DNA(ng/µl) at 4”
100 µl	0.001	ND	ND	ND
	ND	ND	ND	ND
	0.017	0.016	0.001	ND
	ND	ND	ND	0.011
10 µl	ND	ND	ND	ND
	0.006	ND	ND	ND
	ND	ND	ND	ND
	0.002	ND	ND	ND
1 µl	ND	ND	0.003	ND
	ND	ND	ND	ND
	ND	ND	ND	ND
	0.0008	ND	ND	ND
0.1 µl	ND	ND	ND	ND
	ND	0.006	ND	ND
	0.0008	ND	ND	ND
	ND	ND	0.001	ND

Table 1 shows the concentration of DNA (ng/µl) found at the four distances swabbed after collection using the wet-vacuum system. For the most concentrated sample, 100 µl, DNA was detected in 5 out of 16 instances and up to 4 inches away on the surrounding tiles. The average amount of DNA found was 0.01 ± 0.02 ng/µl.

At a volume of 10 µl (Table 1) DNA was detected twice; once at 1 inch and once at 4 inches from the sample tile. The DNA concentration ranged from 0.005-0.001 ng/µl and the average concentration of DNA was 0.001 ± 0.001 ng/µl.

For the next dilution, 1 µl of blood (Table 1), contamination was also found twice; once at 1 inch and once at 3 inches away from the sample tile. The DNA level ranged from 0.004-0.0008 ng/µl and the average amount of DNA was 0.0002 ± 0.001 ng/µl.

At the lowest concentration of blood, contamination was found at three instances; once at 1, 2, and 3 inches from the sample tile. The DNA concentration ranged from 0.006-0.0008 ng/µl and the average level of DNA found was 0.0005 ± 0.0030 ng/µl.

Figure 2a shows the concentration of DNA determined via qPCR plotted against the sample volume at all distances tested. Quantitatively, it is observed that the 100 µl sample resulted in the highest DNA levels collected from the surrounding areas, while the other three volumes resulted in similar concentrations to one another. Further, Figure 2b shows the concentration of DNA plotted against distance. Interestingly, there is no correlation between distance and concentration of extraneous DNA collected: similar levels of DNA were found at 1 inch and 4 inches away from the collection area. This

indicates that small levels of DNA may be detected in areas surrounding a collection site when wet-vacuum technology is used to collect cellular material, and the amount of biological material may impact the level of extraneous DNA found in the surrounding area.

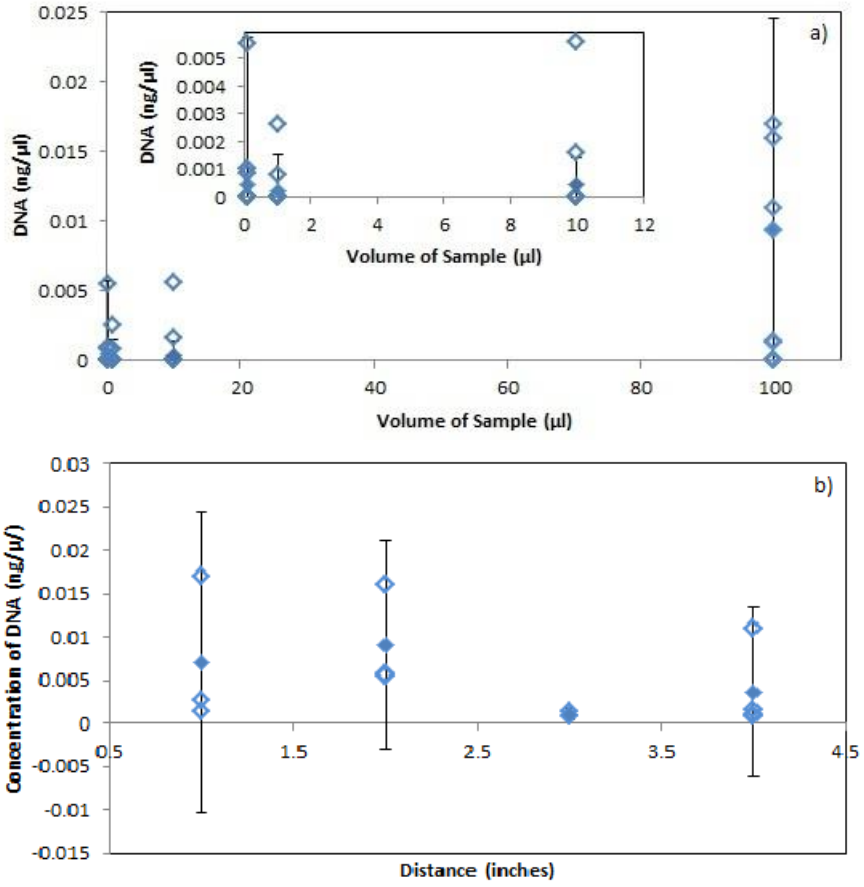

Figure 2: a) The concentration of DNA detected based on the original amount of the blood sample collected at all distances. The inserted graph is a closer visual at volumes 0.1-10 µl. b) Concentration of DNA in ng/µl determined via qPCR plotted against the distance for all volumes. The solid icons represent the average amount of DNA recovered whereas the unfilled icons represent the individual data points

This suggests that on non-porous materials, collection of biological material at any volume with wet-vacuum technology should not be performed in the vicinity of

another sample which has probative value, particularly if adjacent stains are suspected to originate from different contributors. The potential for contamination also suggests that evidence collection using wet-vacuum systems may need to be performed in designated areas where samples from other substrates or cases are not exposed to wet-vacuum collection.

When two probative and separate samples are adjacant to each other, wet-vacuum systems should not be utilized. Rather, swabbing, taping, or cutting is recommended. Further, if there are two adjacent stains, then one would have to be collected using traditional techniques (i.e. swabbing, taping, cutting) while the subsequent collection could be performed using wet-vacuum technology. Alternatively, the application of buffer via pressurization could be omitted and replaced by an independent method of application such as spray bottle or by pipetting the buffer directly onto the sample. This would apply the buffer without significant force, thereby decreasing the possible risks associated with the spraying associated with the vacuum collection system. In addition, this would also allow the forensic laboratory to control the type of buffer used to store the collected samples.

This experiment found sample contamination up to 4 inches away from the edge of the tile containing the sample of interest. The outer limit of the risk of sample spread needs to be determined by further testing.

Further, only tile – a solid, nonporous substrate – was tested. Other types of substrates should be examined, especially those that are commonly encountered at crime scenes, such as carpet, walls, and hardwood flooring (sealed and unsealed), in order to

determine whether this risk is inherent only to non-porous materials and whether porous, absorbant materials also exhibit the phenomenon with wet-vacuum collection.

3.4 Collection by M-Vac® from Substrates

The purpose of developing wet-vacuum collection methods for forensic usage is so samples that would have otherwise been outside forensic examiners' abilities to collect can be gathered for analysis. Samples that fall within this category include, but are not limited to, diffuse samples that are spread over a large surface area, such as a bed sheet or wall. To test the capabilities of wet-vacuum technology to collect samples from a variety of surfaces, blood and semen were spotted onto four substrates (denim, carpet, tile, and brick) at decreasing volumes (100, 10, 1, and 0.1 µl). These samples were then collected with the M-Vac® system, concentrated via filtration, extracted, and then quantified.

3.4.1 Recovery

3.4.1.1 Blood on Tile, Jeans, and Carpet

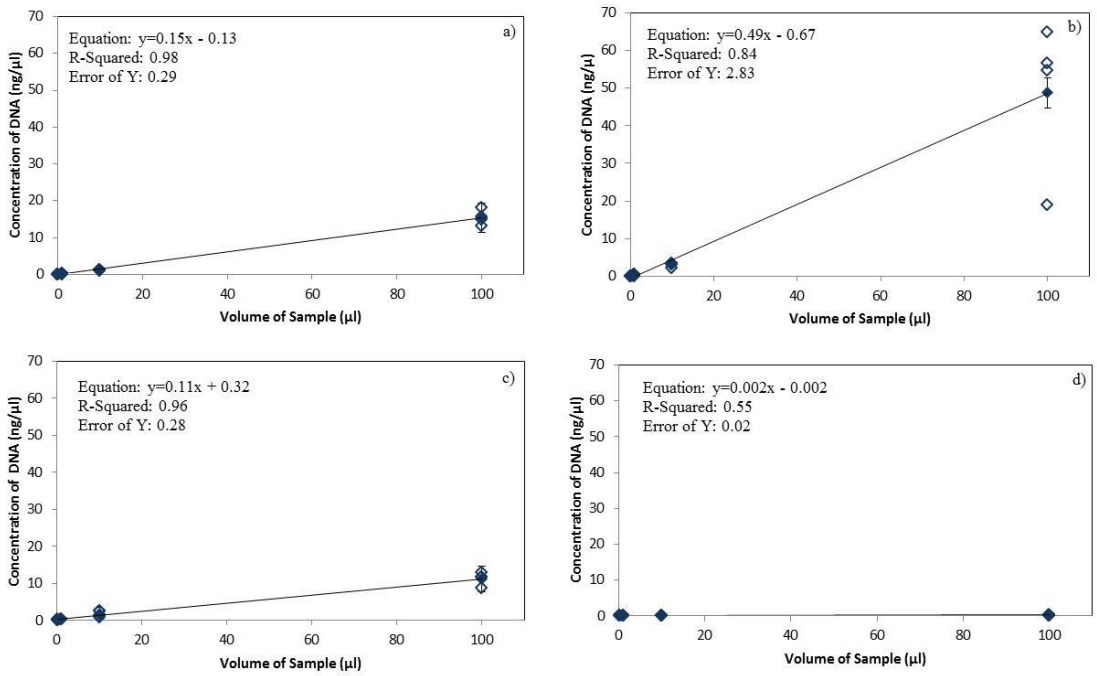

Figure 3: Graphs showing the recovery trends for blood when using a wet-vacuum technology on a) denim, b) tile, c) carpet, and d) brick. The line obtained from brick is essentially parallel with the X-axis, because the recovery amounts were small in comparison with the other substrates. The solid icons represent the average amount of DNA recovered, whereas the unfilled icons represent the individual data points.

Figure 3 shows the linear regression lines for the data sets of all four substrates. Except for the brick substrate, which resulted in nearly no recovery, all substrates showed an $R^2 > 0.8$. If the recovery is assumed to be directly proportional to the volume of biological fluid, then the slope would be an indication of the recovery. The largest slope, hence the highest recovery, was obtained when tile was the substrate, followed by denim and carpet. Therefore for tile and this blood source, an increase of ~0.49 ng/µl of DNA

would be expected for each 1 µl of blood deposited. This drastically decreases when denim and carpet are the substrates and is ~0 ng/µl when brick is the substrate of interest.

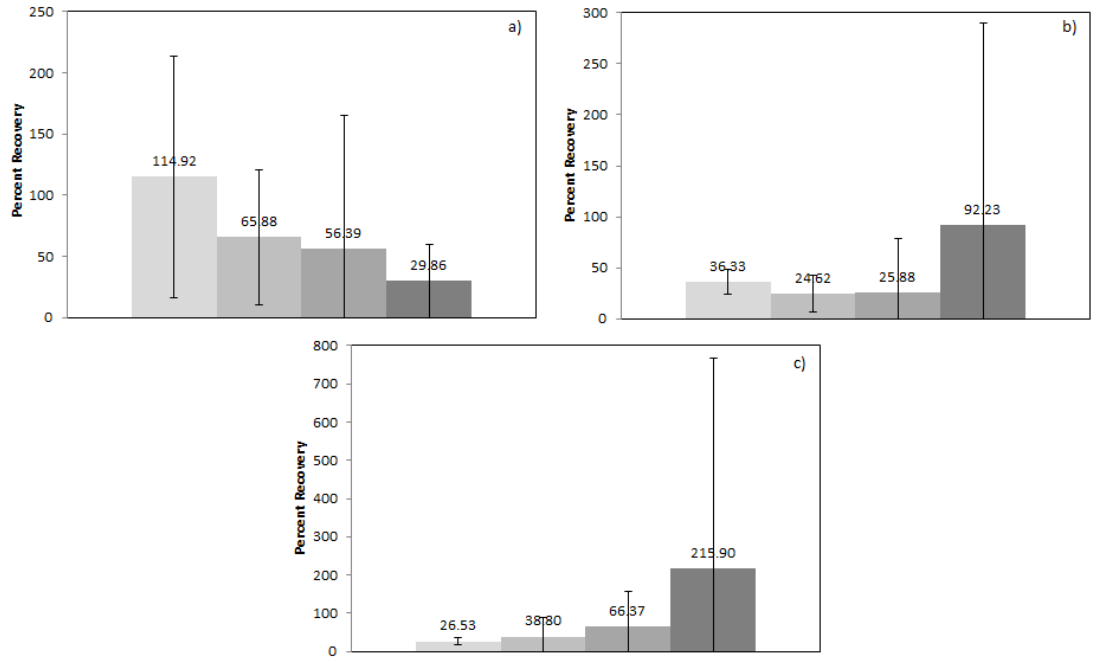

Figure 4: Graphs of percent recovery for a) tile, b) denim, and c) carpet.
0.1 µl 1 µl 10 µl 100 µl

Tile, being smooth and nonporous, shows the highest overall recovery rate. Figure 4 shows the percent recovery as described in section 2.1.4.1 of Materials and Methods for tile, carpet, and denim, and indicates that although percent recovery can vary significantly between substrate types, no general trends were observed with respect to volume. For example, the percent recovery on tile decreased with decreasing sample volume, while it increased when carpet was the substrate. Tile is the only substrate where the raw yield and percent yield increased with the volume of blood collected.

In contrast, the percent recovery increased with decreasing volume when the samples were collected from denim and carpet. The large variability of percent recovery

between samples and substrates, render comparisons between volumes difficult. However, the relative standard deviations (%RSD) calculated via

$$\%RSD = \frac{\sigma_{\%Recovery}}{\%Recovery} \quad \text{(Equation 2)}$$

where $\sigma_{\%Recovery}$ is the standard deviation and $\%Recovery$ is the mean percent recovery, over the replicates show that volume may have an effect on the ability to obtain consistent DNA collection, and consistent DNA extraction is difficult as stochastic processes associated with each DNA processing step propagates. For example, the %RSD for tile is 43% and 50% for 100 and 0.1 µl, respectively. Similarly the %RSD for denim is 16% and 107% for 100 and 0.1 µl, respectively. Carpet also resulted in a larger %RSD when 0.1 µl was collected.

Carpet and denim show similar recovery trends when compared to the raw yield. Unlike tile, denim is expected to be absorbent and the carpet, which was a standard runner made of synthetic fibers, was also expected to be more absorbent. During sample deposition, both seemed to readily absorb the liquid blood samples.

Unlike tile, denim and carpet both show the trend of having increasing raw yields in comparison to the volume collected, but decreasing percent recovery (Figures 3 and 4).

Denim and carpet were also the two substrates that had residual DNA present in the substrate blanks collected. This level of DNA was minute, with a range of 0.0058 – 0.0011 ng/µl. So that only DNA from the sample volume was used in calculations, the average of the residual DNA was subtracted from the quantified amounts for all denim and carpet samples. The complications of working with brick will be discussed

separately since this substrate warrants extra consideration when used in conjunction with a wet-vacuum collection system.

A previous work which also studied collection via the wet-vacuum technique utilized Amicon® DNA concentration filters as the concentration mechanism, so it is not possible to directly compare the DNA yields from their study to the data generated by these experiments. However, these studies corroborate the results found during this study, suggesting that wet-vacuum collection is capable of successfully recovering DNA from a variety of substrates (27).

Further, data from the Garrett study can be compared directly to the data from this study, since both utilized the same concentration and extraction methods. The data concerning the collection abilities of wet-vacuum collection from tile in Garrett also shows the tile (a non-porous substrate) resulted in higher recovery rates than the porous materials (denim and carpet). Additionally, Garrett also showed large variations in percent recoveries (28). These large variations have also been observed in studies by Phillips (36) which focused on determining the percent recoveries of different extraction methodologies, indicating that the large variations are not due to the collection techniques, but to the expected variation associated with DNA extraction and quantification/amplification.

3.4.1.2 Semen on Tile, Jeans, and Carpet

The recovery of blood using the wet-vacuum technique was successful on various substrates. However, it was of interest to determine whether collection of semen from the substrates tile, denim, and carpet, show the same trend (Figure 5). Generally, it was

observed that the DNA yields for semen are higher than the DNA yields for blood, which was expected since there are more spermatozoa cells than blood cells in equal volumes of the two, resulting in more DNA on average (37). This is quantitatively seen via the slopes of the lines which all resulted in slopes >0.8 (Figure 5). Interestingly, unlike the blood samples, the slopes between tile and the porous substrates did not significantly change. For example, the slopes were 1.07, 1.00, and 0.81 for tile, denim, and carpet respectively. That is, calibration sensitivity of the carpet decreased by only 25% when semen was the sample, while it decreased by a factor of when blood was the fluid of interest.

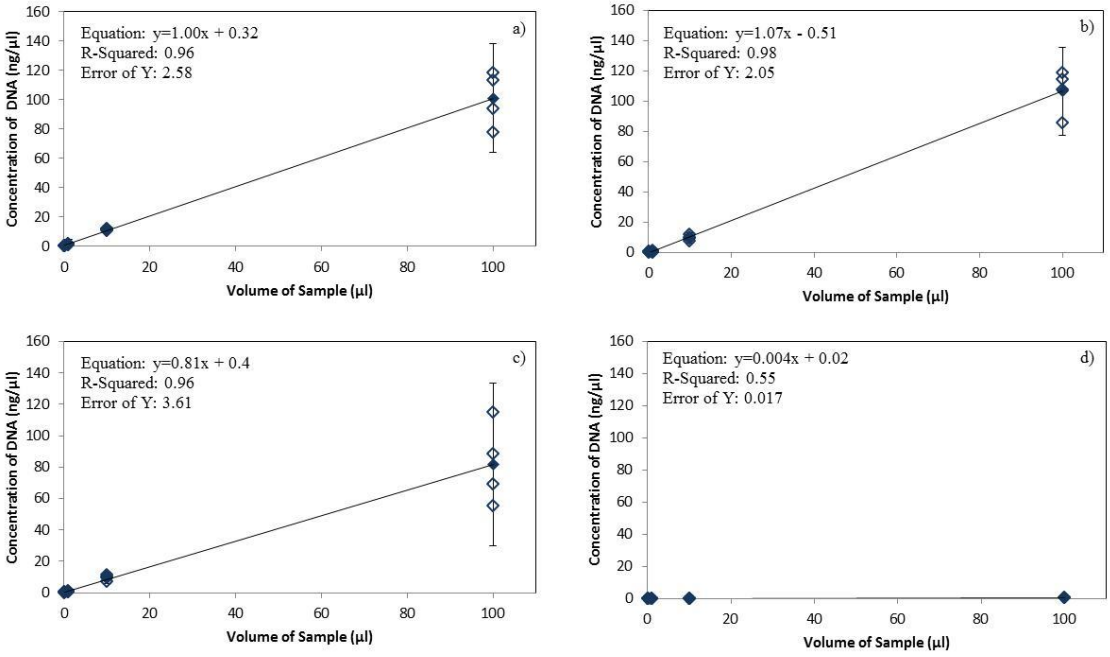

Figure 5: Graphs showing the recovery trends for semen when using a wet-vacuum technology on a) denim, b) tile, c) carpet, and d) brick. The line obtained from brick is essentially parallel with the X-axis with this Y-axis scale, because the recovery

Also, when examining the percent recovery between the two body fluids, the mean percent recovery values associated with semen show a consistency between volumes not seen when blood was the sample. Overall, the semen samples showed a higher level of DNA recovered from all substrates (Figure 6). Further, the %RSD's obtained with 100 µl of semen were 32%, 34%, and 43% respectively for tile, denim, and carpet. In the case of tile, the %RSD is lower, which indicates that there is less variability with semen than with blood, which was 43%.

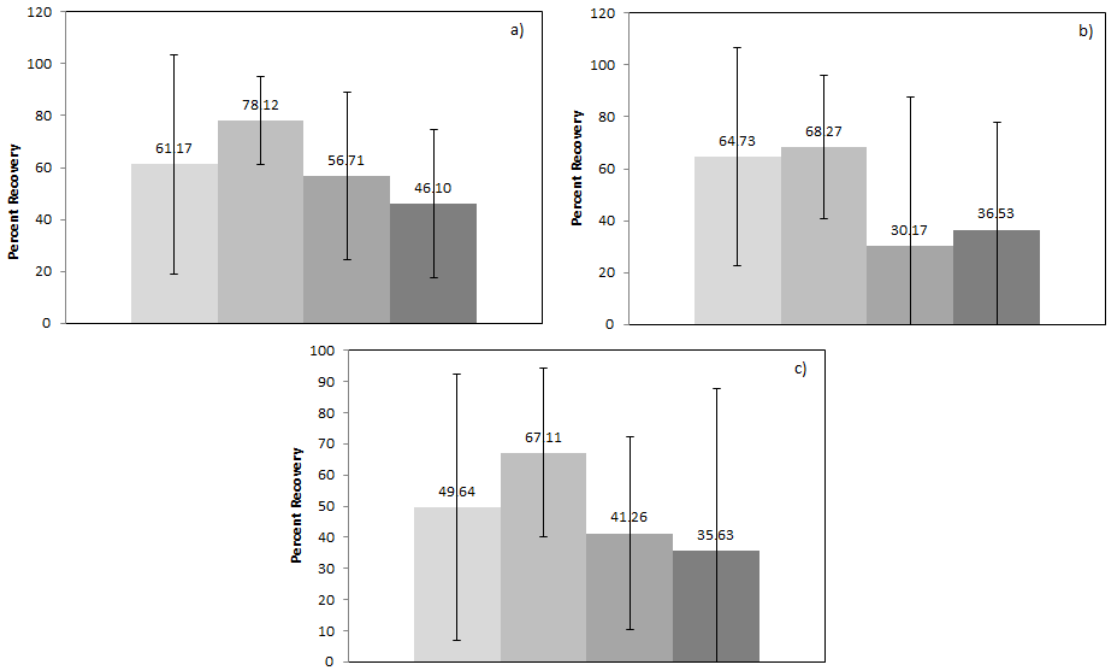

Figure 6: Graphs of percent recovery for a) tile, b) denim, and c) carpet.
0.1 µl 1 µl 10 µl 100 µl

3.4.1.3 Brick

Highly porous brick has traditionally been considered a difficult substrate from which to collect samples and has exhibited low levels of recovery. Frawley *et al.* showed that during collection of *B. anthracis* (anthrax) spores from brick via swabbing, the average recovery was only 2 ± 3% (38).

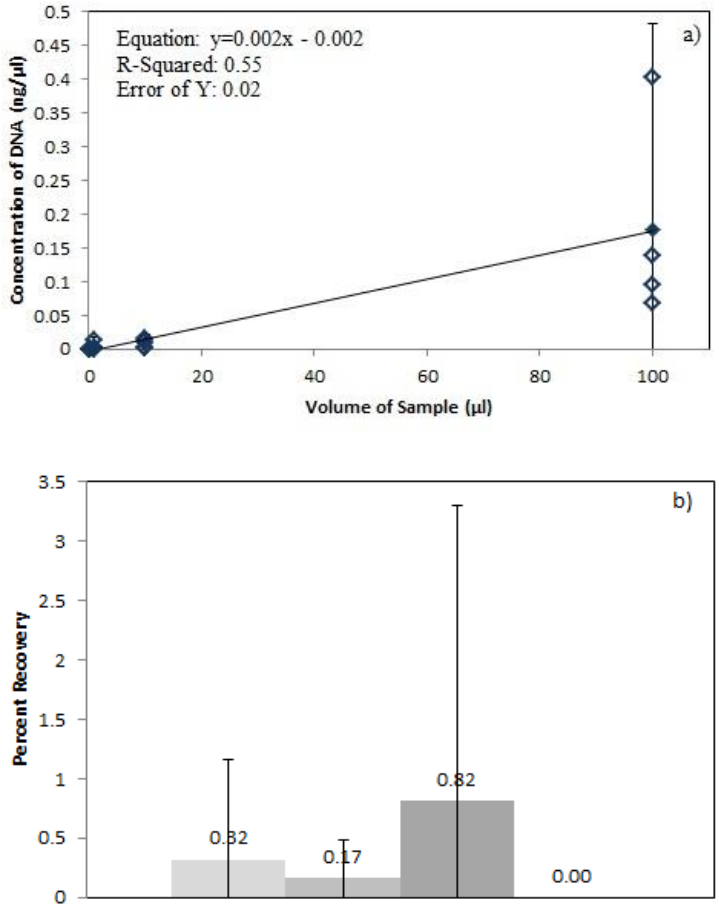

Figure 7: a) Yield of DNA (ng/µl) recovered from blood when collected from brick. The solid icons represent the average amount of DNA recovered whereas the unfilled icons represent the individual data points. b) Percent recovery of DNA from blood when collected from brick. 0.1 µl 1 µl 10 µl 100 µl

Therefore, brick warrants special consideration apart from the other substrates used in this study. When the four volumes of blood were collected from brick, the wet-vacuum collection technique was unable to recover any detectable levels of DNA from the lowest volume of 0.1 µl. The levels of DNA collected from the other three volumes were highly variable, and the sensitivity was 0.002 ± 0.0008 (ng/µl)/(µl) blood indicating that the recovery of brick is sub-par at all volumes tested (Figure 7a).

Further, Figure 7 shows that different volumes of blood on brick do not significantly impact the percent recovery, suggesting that these percent recoveries are up to two orders of magnitude smaller than the recoveries obtained from the other substrates. This shows that collection efficiency is highly dependent on substrate and less so on volume of fluid or fluid type, and low recoveries for brick are expected for all collection techniques – including wet vacuum collection. This is most likely due to the porosity of the brick, which absorbs both sample and buffer into its matrix deeper than the vacuum can retrieve it.

When the M-Vac® was used to collect semen from brick, the general trends of high variability of recovered DNA and lack of trend concerning the percent recovery held true (Figures 8a and 8b). Sensitivity of semen on brick was higher than the sensitivity of blood at approximately 0.30 ± 0.09 (ng/ul)/(ul) semen.

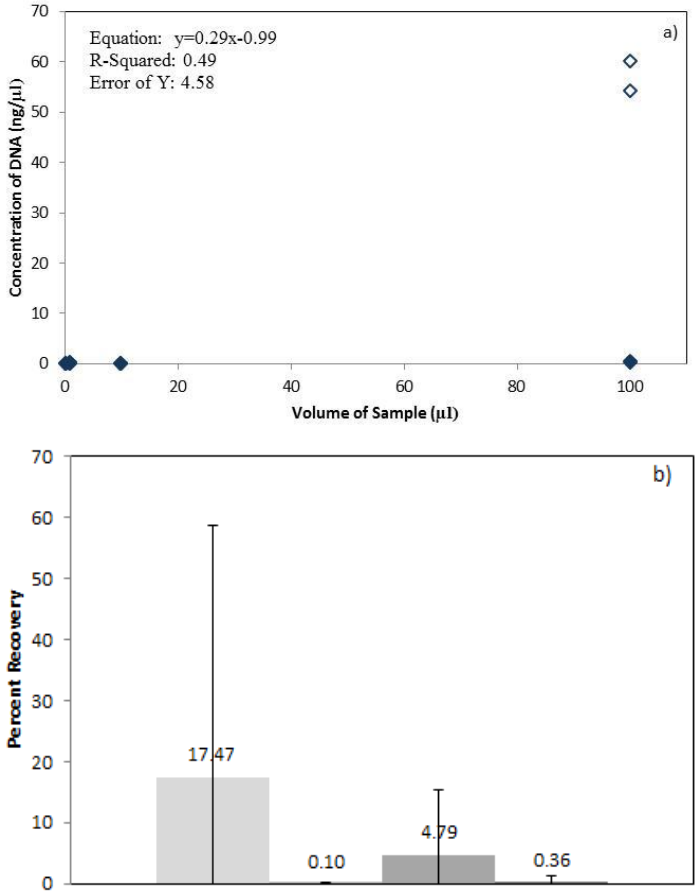

Figure 8: a) Yield of DNA (ng/µl) recovered from semen on brick. The solid icons represent the average amount of DNA recovered whereas the unfilled icons represent the individual data points. b) Percent recovery of DNA from semen when collected from brick. ■ 0.1 µl ■ 1 µl ■ 10 µl ■ 100 µl

It should be noted that there were two data points relating to the recovery of DNA from the 100 µl sample which were potential outliers (Figure 8a). It was observed that the side of the brick used for these two samples did not absorb the sample as quickly as had been the case in all other samples. As a result, these samples yielded more DNA than the samples on the same substrate.

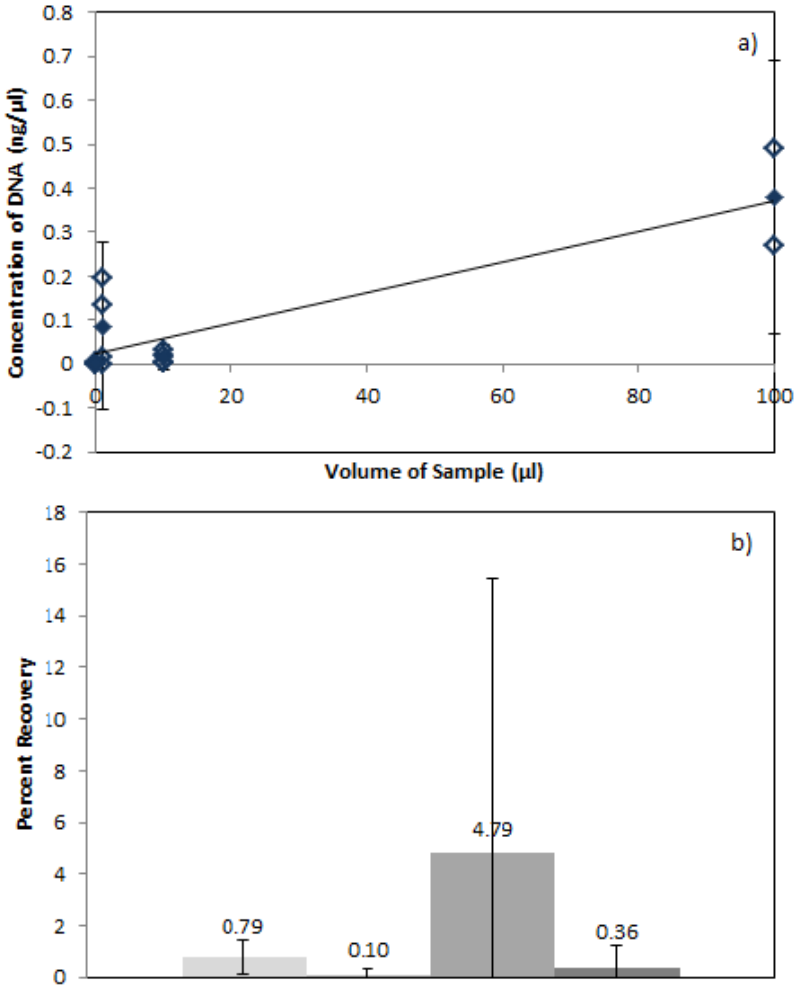

Figure 9: a) Yield of DNA (ng/µl) recovered from semen on brick with the two outlying data points removed. The solid icons represent the average amount of DNA recovered whereas the unfilled icons represent the individual data points. b) Percent recovery of DNA from semen when collected from brick. 0.1 µl 1 µl 10 µl 100 µl

When these two samples are removed from analysis, the sensitivity is approximately 0.03 ± 0.02 (ng/µl)/(µl), which is similar to the sensitivity obtained from the blood on brick. It is possible that this side had a film treatment on it, which prevented the samples from absorbing the cells into the matrix of the brick. When these two points are removed from consideration, the recovery looks similar to the recovery trend expected for such a porous substrate (Figure 9).

In addition, it should be noted that brick is very rough on the M-Vac® collection handset, easily destroying the soft plastic of the nozzle after only a few uses on single bricks. This causes plastic debris to be collected with the sample in the collection bottle.

This debris is then collected onto the filter during concentration and remains in the sample during extraction. Though the plastic seemed to have no negative effect on sample processing, the destruction of the handset requires consideration from a practical perspective.

3.4.2 IPC Analysis

In qPCR amplification, there is an Internal PCR Control (IPC). This control is a specific amount of DNA added to the master mix of qPCR regents. The IPC serves as a positive control and indicates whether the PCR amplification process occurred as expected. When the IPC of a sample varies away from the ideal value, which is between 29-30, it is an indication that there is some agent interfering with the PCR process. This interference is likely an inhibitor, something that prevents the amplification of DNA by somehow interacting with the components of the master mix.

There is a violet or indigo dye occasionally used in the production of dark blue jeans that is a known PCR inhibitor (31). Therefore, the IPC values of all samples were examined to detect possible PCR inhibition. The average IPC values for both biological samples on all substrates were calculated and compared to determine whether PCR inhibition occurred in any instances (Table 2). As seen in Table 2, all IPC's were between 29-30 for all substrates, suggesting no inhibition was observed for any sample set, including denim.

Table 2: Average IPC values and 2 standard deviations from the average for each biological on each substrate.

	Neat	Tile	Carpet	Brick	Jeans
Blood	29.6±0.6	29.4±0.3	29.4±0.1	29.4±0.1	29.4±0.2
Semen	29.5±0.2	29.3±0.3	29.4±0.4	29.2±0.3	29.5±0.3

4. Conclusion

Originally developed to collect microbial cells from carcasses, the M-Vac® Cellular Collection System has the potential to aid crime scene analysts in collecting small amounts of potentially probative cellular materials from large surfaces. The data indicates it has the ability to collect blood and semen from a variety of surface types and managed to recover some DNA from porous brick, a challenging substrate. Since an effective cleaning procedure has also been developed, analysts would be able to reuse handsets for multiple samples and at different crime scenes without risking cross-contamination between samples. The buffer provided by the M-Vac® manufacturer has no noticeable negative effect on the possible cellular recovery when compared to DIH_2O.

However, when choosing between collection techniques, sample-to-sample crossover due to the force at which the buffer is applied to the surface must be considered. In addition to the suggestion that the buffer be administered separately, crime scene analysts would need to ensure that they do not have any samples within at least 4 inches of the area in which they plan to use the wet-vacuum technique, because contamination between samples may occur. Alternatively, they would have to ensure that any samples in the vicinity have already been documented and collected via another technique.

Overall, the wet-vacuum cell collection technique begins to fill the need for more advanced crime scene collection methods for biological samples, particularly samples that may have traditionally been considered impossible to collect; such samples include

diffuse stains spread over a large area. With improvements and optimized protocols, the M-Vac® Collection System could prove a valuable addition to the biological evidence collection techniques currently used.

5. Future Directions

The wet-vacuum collection is capable of collecting low levels of cellular material from a variety of surfaces. However, the instrument and its usage are not without limitations. There is a need for further research and development into wet-vacuum collection and its capabilities, before widespread adoption into forensics.

For example, examinations into how wet-vacuum techniques perform when collecting from a vertical surface, such as a wall, are required. The buffer would be at risk of running down the surface, risking contamination of any area below the collected area and the floor surface, as well as sample loss.

Further, DNA recovery studies examining the efficiency of the wet-vacuum technique to collect biological material from surfaces that have been cleaned with common cleaning reagent (i.e. bleach) would be of interest. The chemicals would be collected along with the cellular material in a higher concentration than on a swab and these reagents may react with other reagents in downstream processes or cause degradation/damage to the DNA.

Finally, other types of biological material should be tested, such as saliva, dry epithelial (skin) cells and urine. Recovery of cellular material from a urine stain is very difficult due to the low level of cells shed from the urethra. Being mostly water, urine

can spread over a large area, making recovery of a sufficient quantity of cells unlikely. Therefore, future studies aimed at confirming the positive results found in this study on diffuse evidence such as urine, wearer, and touch DNA would be of interest.

LIST OF JOURNAL ABBREVIATIONS

Anal Bioanal Chem	Analytical and Bio analytical Chemistry
Cell Mol Life Sci	Cellular and Molecular Life Sciences
Forensic Sci Comm	Forensic Science Communications
Forensic Sci Int	Forensic Science International
Int Congr Ser	International Congress Series
Int J Food Microbiol	International Journal of Food Microbiology
J Applied Microbiol	Journal of Applied Microbiology
J Forensic Sci	Journal of Forensic Science
Method Enzmol	Methods in Enzymology
Nucleic Acid Rec	Nucleic Acid Research
Syracuse Sci and Tech L Rev	Syracuse Science and Technology Law Review
U Cin L Rev	University of Cincinnati Law Review

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Curriculum Vitae

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Education

- 2013 Boston University School of Medicine, Master of Science in Biomedical Forensic Sciences (Anticipated August 2013)
- 2010 Washington State University, Bachelor of Science in Genetics & Cell Biology
- 2007 Edmonds Community College, Associate of Science in Biology

Master's Thesis (in progress)

- Validation of the Microbial Vacuum Cell Collection System for Forensic Purposes.

Publications

- Busch, J.W., Herlihy, C.R., Gunn, L., Werner, W.J. Mixed Mating in a Recently Derived Self-Compatible Population of *Leavenworthia Alabamica* (Brassicaceae). Am J Bot. 2010. 97(6).

Presentations

- Mating Strategies of a Hermaphroditic Plant, Lena Gunn and Jeremiah Busch, oral presentation at the National Ronald E. McNair Conference, November 2009.
- Impact of the SWGDAM Mixture Interpretation Guidelines: Successes, Issues and Suggested Future Directions, Robin W. Cotton, John M. Butler, Michael D. Coble, Catherine M. Grgicak, Charlotte J. Word, Lena E. Gunn, poster presented at International Symposium on Human Identification 2011.

Employment History

- September 2010 – November 2011: Data Analysis Assistant, Dr. Robin Cotton. Boston University School of Medicine.
- May 2009 - January 2010: Undergraduate Research Assistant, Dr. Jeremiah Busch. Washington State University.
- September 2007 - May 2009: Undergraduate Lab Assistant, Dr. Karen Killinger-Mann. Washington State University.

Workshops Attended

- Mass Fatality Planning and Response, March 14-16, 2012. Richmond, VA