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IMPROVING DNA EVIDENCE COLLECTION VIA QUANTITATIVE ANALYSIS: A SYSTEMS APPROACH

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ABSTRACT

When collecting biological evidence from a crime scene, it is important to determine the most effective and robust collection method to ensure maximum DNA recovery. Some common biological collection methods include swabbing, cutting, scraping, and taping. Although these techniques have been a mainstay of forensic analysis, each of these methods have significant drawbacks, which include but are not limited to, the lack of surface area that may be processed, possible co-elution of PCR inhibitors, and non-optimized elution of cells from the substrate into solution. Therefore, a technique designed to optimize biological collection from items of interest, particularly large items, is necessary and not currently available for forensic use.

The field of pathogen testing, like forensic science, also relies on optimized sampling and collection. Recent work in this field suggests the use of a wet-vacuum collection system would be a valuable addition to the already established methods of collection. Generally, this method works by spraying sterile buffer onto a potential sample while simultaneously vacuuming the buffer along with any cellular/nuclear material.

In this study, traditional biological collection methods, including the double swab method and taping, are compared to a wet-vacuum system through the collection of different volumes of blood (0.075 – 75 µL) on tile, denim, and carpet. Before comparing each method, whole blood extractions and quantification of these extracts were performed. To accomplish this, the specified volume of blood was spotted onto the surface of each substrate and dried. The sample was then collected through the use of the double swab method, taping using a 2 x 6 cm^2 piece of BVDA Instant Lifters®, or the wet-vacuum system. An additional 0.00025 – 25 µL of blood was spotted onto each substrate and collected for presumptive testing. After collection, extraction and quantification procedures were performed. Each sample was analyzed in triplicate. In addition, one replicate from each collection, along with substrate controls, were amplified using the PowerPlex® 16 HS System and further analyzed through capillary electrophoresis.

Results demonstrate that successful DNA recovery was obtained with the wet-vacuum system on both non-porous and porous surfaces. Additionally, it outperformed the double swab method and taping, in some cases, when

considering DNA recovery. Specifically, minimum distinguishable signals (MDS) and limits of detection (LOD) were determined for each method on each substrate. The MDS for most samples was 37.8 C_T. However, taping and the wet-vacuum system on denim and carpet resulted in lower MDSs. Collections utilizing the wet-vacuum system on denim had the lowest MDS at 29.6 C_T. For collections performed on tile, the double swab method, taping, and the wet-vacuum system had similar LODs of 14, 13, and 15 nL, respectively. For denim and carpet, the taping method resulted in the lowest LOD of the three methods, while the use of the wet-vacuum system resulted in the highest LOD. The highest calculated LOD was obtained when samples were collected with the wet-vacuum system on carpet, 300 nL, and is suggested to be the result of collecting large quantities of DNA already present on the substrates.

Based on these results, suggestions as to which method to use during collection are presented.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

APC	Aerobic Plate Count
BV	Base Volume
°C	Degrees Celsius
cm	Centimeter
C_T	Cycle Threshold
DI H_2O	Deionized Water
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
g	Gravitational Force
in	Inch
Inc.	Incorporated
IPC	Internal Positive Control
LOD	Limit of Detection
MBS	Minimum Base Signal
MDS	Minimum Distinguishable Signal
mL	Milliliter
mM	Millimolar
mm	Millimeter
M-Vac®	Microbial-Vac®
ng	Nanogram
nL	Nanoliter

PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
R^2	Correlation Coefficient
RFU	Relative Fluorescence Unit
ΔR_n	Change in Fluorescence at Cycle n
RNA	Ribonucleic Acid
SD	Standard Deviation
SEC	Support Equipment Case
SRS	Surface Rinse Solution
STR	Short Tandem Repeat
TE	Tris-EDTA (Ethylenediamine Tetra-Acetic Acid)
µJ	Microjoule
µL	Microliter
µm	Micrometre
UV	Ultra-Violet

Introduction

Some of the most vital evidence found at crime scenes is biological in nature. Common biological sources include, but are not limited to, blood, saliva, semen, and touch DNA. When performed properly, processing this type of evidence can lead to significant linkages between potential victims, suspects, evidence, and the crime scene. The primary step to gathering and analyzing biological material is the collection of the evidence itself. It is only with the use of a proper technique that optimal collection of biological material is possible, which in turn allows for successful downstream DNA processing.

During the collection of biological material from a crime scene, it is imperative to implement the most effective and robust technique to ensure maximum DNA recovery. Some of the most common biological collection techniques include swabbing, scraping, taping, and cutting [1-5]. While these have been the most commonly employed methods, there has been little advancement or improvements in the collection process.

The most typical source of DNA can originate from various biological body fluids. Establishing the presence of these types of evidence is important to determine whether or not further collection is necessary and whether DNA analysis will be probative. Previous research has shown there are certain substances within biological fluids that allow for sensitive and specific identification using a multitude of different types of body fluid identification techniques [6-13].

There have also been advancements in DNA quantification and STR (short tandem repeat) analysis/profiling. In 1985, Jeffreys *et al.* provided the foundation of DNA analysis with a tool for individualization [14]. By utilizing specific simple tandem-repetitive or hypervariable minisatellite regions within the genome, a technique to distinguish humans from one another was developed. This technique allowed for individual human identification, which was something that could not be performed previously. Soon after, the Polymerase Chain Reaction (PCR) was developed and utilized as another invaluable technique within DNA analysis [15]. The PCR technique allows for the amplification of small amounts of DNA. The ability to copy or amplify regions of the genome using PCR is especially valuable when there are only minute amounts of biological evidence found at crime scenes.

Throughout the past two decades, multiple improvements and advancements in molecular biology and DNA analysis have continued to arise. A major development has been the introduction of real-time quantitative PCR (qPCR) [16-24]. With qPCR, forensic analysts are able to detect the quantity of the PCR products with each cycle, thus, providing more sensitive quantity measurements. Real-time PCR differs from end-point PCR because PCR plateauing effects do not influence the quantification. Not only is qPCR human specific, but it is also able to detect picogram levels of both total human DNA and total male DNA present within a sample. With the research that has been performed thus far, and with continuing research, DNA typing processes have

become increasingly specific and sensitive, and are expected to become even more so. Despite the significant advancements in DNA processing techniques, there has been little improvement in the area of biological collection. Since DNA results are dependent on the number of cells collected, the technique used to gather the evidence is of import.

Biological Evidence Collection: Common Techniques and Limitations

As stated previously, there are many types of biological collection techniques currently used by forensic analysts. This includes swabbing, scraping, taping, and cutting [1-5]. While each of these techniques has become a mainstay in forensics, each of them also has their drawbacks.

The swabbing method can either be utilized with a single swab or, alternatively, the double swab method can be performed [1-2]. During single swab collection, a sterile cotton swab is first moistened and then rubbed over the location of the potential biological fluid. The swab is rubbed over the stain with some pressure in a circular fashion in order to collect the maximum amount of sample. Although the moistened swab may be able to successfully collect biological material, there is a chance that the swab may become oversaturated or potentially leave behind residual material [1-2]. To assist in this, and possibly ensure a more thorough collection, the double swab method can be performed. In the double swab method, a sterile cotton swab is moistened and rubbed over the stain, as in the single swab method. However, following the use of the

moistened swab, a second dry, sterile cotton swab is then rubbed over the location of the stain. This second dry swab allows any potential residual biological fluid left behind from the first swab to be collected. The two swabs are then processed together during subsequent DNA analysis.

While swabbing is a typical technique utilized by forensic analysts, and the cotton portion of the swab can easily be added directly to a DNA extraction procedure, it is not a practical method for larger substrates. This is especially true when the biological evidence may be dilute and when the exact location of the biological material is not specified. Another concern related to this method is extraction of the cells containing the DNA from the cotton substrate. Elution of the cells from the substrate is an essential component in the processing of DNA evidence, thus, when performing this method, it may be more difficult to 'pull' the cells from the substrate into solution when dealing with minute levels of sample.

Another common technique utilized during biological evidence collection is scraping [3]. The scraping of a substrate on which biological fluid has been deposited involves the use of a tool, such as a sterile scalpel, spatula, or scissors. The tool is scraped over the area of the stain to release dried particles of the biological material containing the DNA. These scrapings are placed into an appropriate container and swabbed. The swab then undergoes typical DNA processing. In a study performed by Stouder *et al*., it was shown that scraping is a viable and reliable method to obtain DNA [3]. By scraping worn hosiery and t-shirts for potential biological material, in which the debris was placed into a

pillbox and subsequently swabbed, it was found that, generally, greater quantities of DNA were obtained as compared to simply swabbing the worn materials. While this demonstrates the potential advantage of scraping over swabbing, this study also shows a potential disadvantage. That is, when scraping into the pillbox and then swabbing the pillbox for subsequent DNA testing, another transfer step was added to the collection method. When adding extraneous transfer steps within a biological collection, there is greater risk of contamination and/or loss of some of the biological material containing DNA. Another aspect to consider is that, while collection through scraping is not really limited in the area that it can cover, it may not be well suited for dilute stains spread over a large area. This is especially true in cases where the substrate is highly absorbent, in which scraping would only collect the material on the upper surface of the substrate. Ultimately, this could prevent some of the biological evidence from being collected, demonstrating another limitation of the scraping method.

Cutting provides forensic analysts with another biological collection technique. In this method, a small piece of the substrate thought to contain biological material is cut with sterile scissors or scalpel and then placed into a vial or tube. The cutting can then be soaked in buffer to allow for presumptive testing and/or other DNA downstream processes. While this method presents analysts with a quick and easy way to collect evidence, there are some disadvantages when dealing with a stain that is not contained within a small area. Cutting only allows a small amount of the material to be tested and, if the biological evidence

is very dilute and spread across a large area, then this method may not allow for sufficient DNA collection; this could potentially cause a false negative result during subsequent analysis. Another issue can arise when cutting a substrate that may contain PCR inhibitors. The potential for co-elution of PCR inhibitors could negatively affect further DNA analysis and, while advancements to deal with the possible effects of inhibitors have been developed, DNA profiles resulting from amplifications that have been inhibited make DNA interpretation difficult [25-27].

A fourth common mechanism of collection is taping [4-5]. This method involves the use of a piece of tape in which the adhesive portion is continually placed and lifted over the area containing a potential biological stain. The use of this method is dependent on the type of tape utilized for collection and the stickiness of the adhesive. While tape may be able to cover larger areas, it is entirely dependent upon how long the adhesive will continue to stick to the substrate and successfully gather the biological material. In turn, this may actually limit the amount of substrate that can be taped. An advantage of taping, unlike swabbing and cutting, is that there is a decrease in the uptake of potential PCR inhibitors. This has been shown by Barash *et al*., where DNA amplification of samples collected with tape was successfully performed on substrates that are commonly known to contain PCR inhibitors, such as denim and leather [4]. According to Li and Harris, tape also provides a collection method that could

decrease degradation of the DNA over time due to the lack of moisture and potential for bacterial growth [5].

While these biological collection techniques have become customary, each of them has obvious shortcomings. These include, the inability to sample large surface areas, elution of cells from a substrate into solution for further processing, and co-elution of PCR inhibitors. Each drawback has the potential to affect presumptive testing, amplification, and STR analysis. There is an obvious lack in advancement within the area of biological collection, resulting in a need for new techniques. Improvements in this area will allow for better optimization in the actual collection process from different items of interest, particularly with larger items. In 2005, Petricevic *et al*. performed a study demonstrating whether trace DNA could be collected and analyzed from bed sheets [28]. Although it was shown that trace DNA could be successfully collected, quantified, and amplified from cuttings taken from the bed sheets, a large substrate, this was not the case for every sample. There were instances in which there was not a sufficient amount of DNA collected to continue with downstream DNA processing. This further presents a need for developments to be made to ensure that analysts are able to more effectively collect biological evidence from large substrates.

New Collection Technique: A Wet-Vacuum Collection Technique

A possible alternative to typical biological collection methods that may address some of the aforementioned issues is the use of a wet-vacuum system.

This tool is designed to collect samples through the use of an output of a sterile solution onto a substrate of interest, while simultaneously vacuuming this solution - along with potential biological material - into a sterile collection bottle. For example, one such commercially available wet-vacuum system is the Microbial-Vac® collection system, or M-Vac® (Microbial-Vac Systems Inc., Bluffdale, UT). This system consists of the following: (1) The Support Equipment Case (SEC) 100 Unit containing the pressurization chamber for the sterile surface rinse solution (SRS), the vacuum system, and airflow tools; (2) Sterile Surface Rinse Solution (SRS), packaged in solution bags, which is administered with pressure onto the surface of the substrate of interest and subsequently vacuumed - along with any potential biological material; (3) M-Vac® kits, which are disposable sampling devices utilized for sterile collection and include the collection headset, allowing the output of the sterile SRS with subsequent vacuuming, filtered chambers, and a sterile collection bottle; (4) SEC extension tubing, allowing the M-Vac® Kits to be connected to the SEC; (5) Sterile M-Vac® collection bottles in which the SRS and any biological material are collected and retained until further testing ensues.

The use of a wet-vacuum technique introduces some advantages when compared to other common techniques. Due to the use of a sterile solution being sprayed onto the surface of the substrate, pressure and aggravation to the stain is applied. This may in turn assist in increasing the amount of biological material ‘pulled’ from the substrate. Another potential asset of this technique is that it

essentially has no limit with respect to the area that it can sample. Not only does this allow analysts to overcome the limitations of aforementioned collection techniques, but it would also allow for large substrates to be more efficiently processed, especially in cases where the biological evidence is dilute and not localized to a confined area. The use of this technique could present forensic analysts with an efficient substitution to other methods and, ultimately, assist in overcoming some of the drawbacks associated with traditional collection techniques.

Research performed in the field of pathogen testing suggests the wet-vacuum system may be a viable alternative to already established collection methods. In recent work performed by Bradley *et al.*, the M-Vac® system was compared to a common sampling method used in pathogen testing - the sponge method [29]. Between the two methods, 24 samples were collected from meat carcasses; specifically from adjacent sites of brisket, flank, and rump. Overall, it was observed that the wet-vacuum system resulted in higher Aerobic Plate Counts (APC) - a metric of the number of microbes found - than the sponge method in all cases. The average APC for the M-Vac® was $\log_{10}$ 3.91 ± 0.51 while the average APC for the sponging method was $\log_{10}$ 3.11 ± 0.57 ($P \leq 0.05$). It was also observed that the M-Vac® filters collected low levels of *E. coli* in 8 of 12 samples [29]. The success of the M-Vac® in this study suggests that wet-vacuum collection may be a valuable addition/alternative to other collection techniques used in the field of forensic science.

Further, a study performed by Sorenson Forensics (Salt Lake City, UT) compared the collection of blood and saliva samples from white cotton, blue denim, polyester, and nylon using the M-Vac®, swabbing, or cutting methods [30]. Specifically, in experiments comparing the swabbing method and the M-Vac®, it was reported that the use of the wet-vacuum technique yielded higher levels of DNA, as per qPCR, than the swabbing method for samples of blood and saliva. It is important to note that there was a higher yield of DNA detected with the wet-vacuum technique than with neat saliva samples, however, there was no explanation as to why this may have occurred. Despite this, the results from this preliminary research, especially when compared to swabbing, indicate that this technique may be a valuable addition to other collection methods and may also be a useful tool during crime scene processing.

Other research has addressed practical issues related to this instrument. Specifically, Johnson compared potential concentration methods in order to consolidate the cellular/nuclear material collected from a sample contained in 250 mL of collection buffer [31]. It was shown that a filtration method allowed for better sample concentration than a method based on evaporation. If using a wet-vacuum collection system, this filtration method would allow for the concentration of larger sample volumes, which is particularly important if collecting from large surface areas. Further, Gunn developed a cleaning method for the headsets and connected tubing of the instrument, enabling reuse of these parts, and also determined that the M-Vac® was successfully able to collect DNA from samples

of blood and semen from a variety of substrates including tile, denim, carpet, and brick [32]. Although the wet-vacuum system was efficiently able to collect DNA from these substrates, it was also shown that the force of the buffer might have caused some sample carry-over near the location of the sampling area, where positive DNA results were detected up to 4 inches away from the collection area. This was attributed to the applied force of the buffer onto the substrate [32].

Purpose

The purpose of the following research was to compare traditional biological collection methods to a wet-vacuum collection system. More specifically, the double swab method and taping method were compared to the M-Vac® system (Microbial-Vac Systems Inc., Bluffdale, UT) through the collection of different volumes of blood (0.075 – 75 µL) on tile, denim, and carpet. In a separate set of experiments, preliminary testing on 0.00025 – 25 µL of the same blood samples was also performed [33].

After collection with each of the methods, each sample was subjected to DNA extraction using the QIAamp® Investigator extraction protocol (Qiagen, Valencia, CA) and DNA quantification using the Quantifiler® Duo Quantification Kit (Applied Biosystems, Foster City, CA) and the 7500 Detection System (Applied Biosystems, Foster City, CA). In addition, one replicate from each collection set and the substrate controls were amplified using the PowerPlex® 16 HS System (Promega, Madison, WI) and further analyzed through capillary

electrophoresis using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) and the GeneMapper® ID-X Software (Applied Biosystems, Foster City, CA).

The results obtained were then compared and analytical figures of merit were calculated to assess the efficacy of collecting biological material from a specified substrate using each technique. The STR profiles obtained from select samples were used to determine the minimum number of contributors for each profile and the average peak heights of the substrate controls. These results were then applied to evaluate which of these biological collection methods is recommended based on the circumstances and nature of the biological evidence of interest. Final recommendations are provided in a flow-chart.

Materials and Methods

All aspects of this study were conducted in compliance with ethical standards set forth by the Institutional Review Board of Boston University School of Medicine, Protocol H – 26187.

Unless otherwise stated, all reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of Blood Samples

Blood dilutions were prepared and used throughout the study. The dilutions of blood consisted of whole blood, a 1:10, a 1:100, and a 1:1000 dilution. A negative control was also prepared and showed expected results.

For each dilution, the appropriate amount of blood and TE (Tris-EDTA; Ethylenediamine Tetra-Acetic Acid; $1x10^{-4}$ mM) buffer was pipetted into a labeled microcentrifuge tube and mixed. A total volume of 4300 µL was made for each dilution. See Table 1 for a summary of the blood samples prepared.

Table 1. *Preparation of blood dilutions.*

Sample Name	Volume of Blood (µL)	Volume of TE Buffer (µL)	Total Volume (µL)	Dilution Description
B-200	4300	0	4300	Whole Blood
B-201	430	3870	4300	1:10
B-202	43	4257	4300	1:100
B-203	4.3	4295.7	4300	1:1000
B-PB-053112-AG	0	4300	4300	Negative Control for Preparation of Samples

Preparation of Substrates

A total of three substrates were used for collection including a non-porous substrate, tile, and two porous substrates, denim and carpet. For each blood dilution, three replicates were created per substrate.

Ceramic tiles were utilized for the collection of samples on a non-porous surface. Before spotting each dilution, the tiles were cleaned with 10% bleach, DI H_2O (deionized water), and 70% ethanol, respectively. For collections using the double swab and taping methods, 75 µL of the appropriate blood dilution was spotted onto one half of the dry tile. Another 25 µL was spotted onto the other half of the substrate to be used in a separate set of presumptive testing experiments [33]. For the wet-vacuum collections, the commercially available M-Vac® (Microbial-Vac Systems Inc., Bluffdale, UT) was used and 100 µL of the appropriate blood dilution was spotted onto each tile, in which only 75% of the collection was used for further DNA analysis. Each sample spotted on the tiles was allowed to dry for approximately three hours prior to collection.

For collections performed on denim and carpet, cuttings were created for each blood dilution to be spotted (approximately 7 cm^2). Before use, UV irradiation was performed on each cutout using a Spectroline® XL-1500 Crosslinker (Spectronics Corporation, Westbury, New York). Both sides of the denim and carpet cutouts were UV irradiated at 3000 x 100 µJ/cm^2 according to the protocols suggested by Spectronics Corporation [34-35]. Like the tile, 75 µL (and an additional, separate 25 µL) of the appropriate blood dilution was spotted

onto the substrate for the double swab and taping methods, and 100 µL was spotted onto the substrate for the wet-vacuum collections. Again, each sample was allowed to dry for approximately three hours before collection.

In conjunction with the collection of blood dilutions, a non-stained substrate was used as a substrate control. This blank substrate was cleaned or UV irradiated according to the above protocols; however, no blood was spotted onto the surface. Collection and other analyses on these substrates were performed in the same manner as all other samples collected.

Collection of Blood Samples Using the Double Swab Method

The first biological collection technique performed was the double swab method [1-2]. A volume of 50 µL of DI H_2O was pipetted onto a sterile cotton swab. This swab was then rubbed over the surface area of the substrate where the blood sample was located. During this process, the swab was rotated. After the use of this wet swab, a second, dry, sterile cotton swab was subsequently rubbed over the area where the sample was located. Using the same technique as the first swab, this second swab was also rotated during collection over the area of the sample.

The swabs were allowed to dry overnight before being stored and/or performing DNA extraction procedures. For each collection performed, a substrate control was also collected on each type of substrate with no sample added to the surface.

Preliminary Tape Experiment: Choosing a Tape

To determine which kind of tape would be utilized for all tape collections, preliminary testing was performed to ensure that the specific tape used would go through the QIAamp® Investigator extraction protocol (Qiagen, Valencia, CA) successfully.

The first tape tested was Scotch® Brand Mask Plus II Water Soluble Wave Solder Tape (3M™) (3M, St. Paul, MN). To begin, different size pieces of tape were cut including a 10 cm x 1.9 cm, 7 cm x 1.9 cm, 5 cm x 1.9 cm, 3 cm x 1.9 cm, and a 1 cm x 1.9 cm piece. Before completing the extraction procedure, each piece of tape was UV irradiated at 3000 x 100 $\mu J/cm^2$ on both sides. This was done to determine whether the UV irradiation would potentially affect the tackiness of the adhesive portion or the tape's ability to be used during extraction. During the extraction process, it was observed that the QIAamp® MinElute columns utilized became clogged with the adhesive, with the exception of the 1 cm x 1.9 cm piece of tape, preventing the full extraction procedure to be completed. Because it would not have been practical to use this small size for collection of the samples, a different type of tape was needed for the Qiagen extraction procedure.

The second tape tested was BVDA Transparent Instant Lifters® (BVDA, Haarlem, The Netherlands). Like the Water Soluble Wave Solder Tape, the BVDA Instant Lifters® were first cut into different size pieces including an 8 cm x 2 cm, 6 cm x 2 cm, 4 cm x 2 cm, and a 2 cm x 2 cm piece. Before extraction,

each piece was UV irradiated at 3000 x 100 μJ/cm^2 on both sides. Due to the lack of flexibility of the BVDA Instant Lifters®, each piece of tape was cut into small pieces with cleaned scissors before being placed into a microcentrifuge tube to continue with the extraction procedure. It was observed that all sizes of the BVDA Instant Lifters® could be used in the extraction procedure and did not cause clogging of the QIAamp® MinElute columns. Due to the size of the samples to be collected and for better ease of use, it was decided that the 6 cm x 2 cm piece of BVDA Instant Lifters® would be utilized for all sample collections.

Collection of Blood Samples Using Tape

In order to utilize the full size of the tape pieces for sample collection, the tape was first cut into 8 cm x 2 cm pieces. At 1 cm from each end of the piece of tape, a small slit was made. These 1 cm flaps were used to hold each piece of tape during collection so as to collect each sample with the full 6 cm x 2 cm tape piece. Before each collection, both sides of the pieces were UV irradiated at 3000 x 100 μJ/cm^2 to ensure sterilization before being placed onto the substrates. This was done by adhering the 1 cm flaps on each side of the piece of tape to a small weigh boat. By placing the flaps to the weigh boats, this would prevent the adhesive portion of the tape, to be used for collection, from coming into contact with any other surface beforehand.

After the samples spotted on the substrate dried and the tape was UV irradiated, the tape pieces were held on each side using the 1 cm flaps and then

carefully pressed against the area of the substrate where the sample was placed. Each piece of tape was pressed against the substrate 20 times. The tape was then placed into a clean weigh boat and covered. The tape was allowed to sit overnight before extraction procedures were performed. Like with the double swab method, a substrate control was collected on each type of substrate using the BVDA Instant Lifters®.

Preliminary M-Vac® Experimentation: Centrifugation vs. Vacuum Filtration

When using the wet-vacuum system, a large amount of buffer is used during collection to extract the sample from the substrate. Therefore, before using this instrument, it was important to determine how each sample collected would be concentrated in order to proceed with the extraction procedure. In determining what method to use, a centrifugation method and a vacuum filtration method, as developed by Johnson [31], were compared. For this comparison, samples of whole blood and a 1:10 blood dilution were prepared. To begin, M-Vac® collection bottles were cleaned, see Figure 1, and then 100 mL of the M-Vac® buffer (SRS) was added to each labeled bottle. For each sample, 100 µL of whole blood or the blood dilution was added to the bottles and gently mixed. Three replicates of each dilution were prepared for both the centrifugation method and the vacuum filtration method.

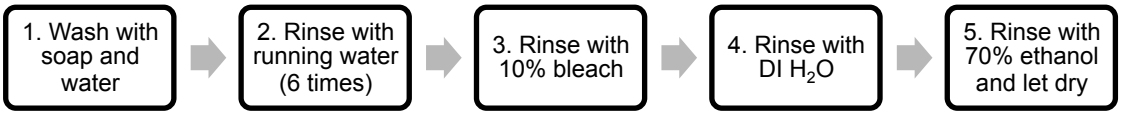

Figure 1. *Outline of cleaning method for M-Vac*® collection bottles.

For the centrifugation method, approximately 50 mL of the appropriate solution was placed into a labeled 50 mL centrifuge tube. The tube was then centrifuged at 10,015 x g for 15 minutes. After centrifugation, the supernatant was removed and placed into an appropriate waste receptacle. This process was repeated with the remaining 50 mL of solution in the M-Vac® bottle. After discarding the remaining supernatant, the QIAamp® Investigator extraction procedure (Qiagen, Valencia, CA) was followed [36].

For the vacuum filtration method, the dilutions were prepared in the M-Vac® bottles as described above. This vacuum filtration method is based on previous research [31] and uses the Millipore™ vacuum filtration system (Millipore, Billerica, MA). Before performing this method, all glassware was cleaned with 10% bleach and 70% ethanol, and the system was assembled according to the manufacturer's recommendations [37]. Using Millipore-Durapore® 0.45 µm membrane filters with a filter diameter of 47 mm, each blood dilution prepared was filtered through the apparatus. To ensure all blood cells containing the DNA were deposited onto the filter, the sides of the collection bottle were rinsed with DI H_2O and this solution was then poured into the funnel system. The funnel walls were also rinsed using DI H_2O, making sure not to

spray directly on the filter. After all solution was permeated through the system, the filter containing the trapped cellular material was then removed, cut with clean scissors into small pieces, placed into a microcentrifuge tube, and run through the extraction procedure. It is important to note that the apparatus was thoroughly cleaned with 10% bleach and 70% ethanol after each collection to prevent DNA carry-over. For the vacuum filtration method, a cleaning blank was also collected using a sterile swab moistened with DI H_2O that was rubbed across the glassware, focusing on the areas where the DNA may have come into contact with.

After the QIAamp® Investigator extraction protocol (Qiagen, Valencia, CA) was performed for each collection and the cleaning blank from the filtration method, quantification was performed using the Quantifiler® Duo Quantification Kit (Applied Biosystems, Foster City, CA). After quantification, it was determined that the filtration method resulted in higher DNA recoveries. Specifically, the average concentration of the samples extracted after concentration using centrifugation was 59 ± 21 ng/μL for whole blood and 1.3 ± 1.2 ng/μL for the 1:10 blood dilution. For the vacuum filtration method, the average concentration of samples extracted was 72 ± 16 ng/μL for whole blood and 6.9 ± 3.0 ng/μL for the 1:10 blood dilution. From these results, it was determined the vacuum filtration method recovered higher concentrations of DNA than the centrifugation method and was chosen as the concentration technique for samples collected by the wet-vacuum system.

Preliminary M-Vac® Experimentation: M-Vac® Collection Procedure

To determine how the samples on each substrate would be collected with the M-Vac®, an experiment was performed to decide how the M-Vac® headset would be used on the sample in order to ensure the most DNA recovery. The first experiment involved using little movement of the M-Vac® headset over the sample area on the substrate. Essentially, the headset was localized around the area where the sample was spotted and was not moved around the entire area of the substrate. The second experiment involved increased headset movement over the sample and the substrate. With this method, the headset was localized around the area where the sample was spotted and then it was moved around the entire surface area of the substrate.

To perform these experiments, samples of whole blood and a 1:10 blood dilution were used and the substrates consisted of tile and denim. On each prepared substrate, 50 µL of the appropriate dilution was spotted, without spreading, and was allowed to dry for approximately three hours.

Before collection, the M-Vac® system was prepared and set-up making sure the buffer solution was placed in its chamber and all tubing was connected. For reference, Figure 2 provides a visual representation of a wet-vacuum system tool similar to the one utilized in this study.

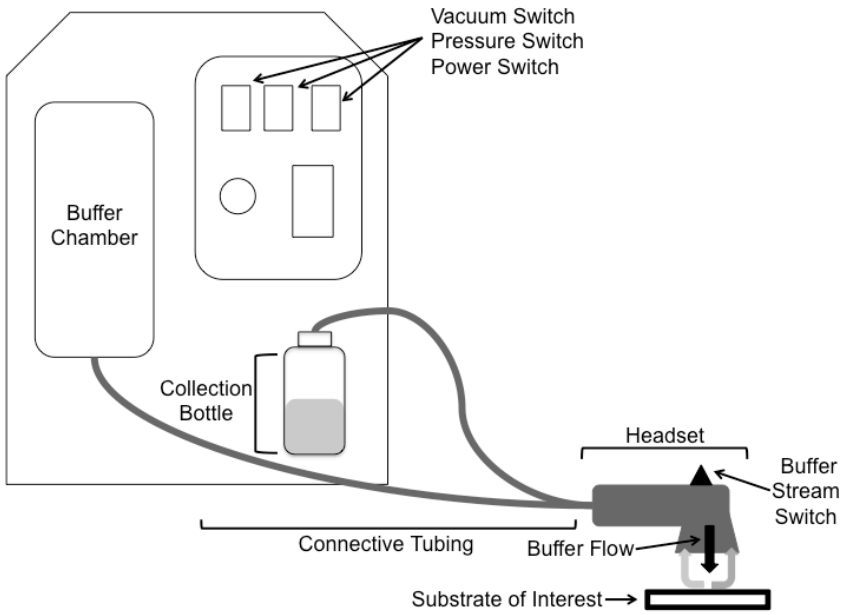

Figure 2. *Visual representation of the wet-vacuum system used for sample collection.*

When set-up was complete, the M-Vac® was turned on, and the pressure was switched on in order for the buffer to stream steadily through the system when in use. Prior to sample collection, the tubing of the M-Vac® was cleaned to ensure no cross contamination. During this procedure, the switch on the M-Vac® headset allowing the flow of buffer through the system was switched off. To begin, 100 mL of DI H_2O in a beaker was vacuumed through the tubing by turning the vacuum switch to the on position. Next, 500 mL of 10% bleach was vacuumed through the tubing, followed by 250 mL of 70% ethanol. To complete this process, an additional 100 mL of DI H_2O was vacuumed. After the cleaning process for the tubing was performed, the outside of the collection headset was wiped using 10% bleach followed by 70% ethanol. This cleaning method was performed before and after all sample collections and was validated through research

performed by Gunn [32]. Figure 3 is an outline of the cleaning procedure. The M-Vac® collection bottles to be used were also cleaned according to Figure 1.

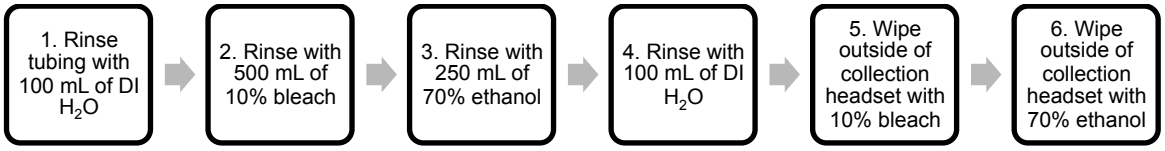

Figure 3. *Outline of cleaning method for the M-Vac*® headset as developed by Gunn [32].

The M-Vac® headset was then placed onto the substrate at a 90° angle. Following this, the vacuum suction was turned on while simultaneously positioning the solution buffer knob (buffer stream switch), on the top of the headset, to its on position, allowing the SRS to be deposited onto the substrate. Using the M-Vac® with little headset movement, 100 mL of buffer was used to collect each sample. For this procedure, the M-Vac® headset was localized around the area where the sample was located. For collection with increased M-Vac® headset movement, 100 mL of buffer was used to collect each sample, however, the headset was first localized around the sample area and then moved across the entire substrate surface, which was approximately 4.25 in^2 for both tile and denim substrates. For each procedure, three replicates were analyzed. After collection and vacuum filtration, extraction and quantification procedures were performed.

Overall, it was observed that the M-Vac® collections performed with little movement of the M-Vac® headset recovered more DNA than the collections

performed with increased M-Vac® headset movement, particularly when denim was the substrate. Average DNA concentrations (in ng/µL) recovered for each procedure are summarized in Table 2. Each error presented represents two times the standard deviation.

Table 2. *DNA concentrations ± 2SD recovered from tile and denim with varying M-Vac®* headset movement.

Substrate	**Whole Blood Recovered (ng/µL)**		**1:10 Blood Dilution Recovered (ng/µL)**	
	Little Headset Movement	**Increased Headset Movement**	**Little Headset Movement**	**Increased Headset Movement**
Tile	33 (± 7)	36 (± 6)	3 (± 2)	1 (± 2)
Denim	34 (± 8)	18 (± 13)	2 (± 2)	1.7 (± 0.5)

As a result, all subsequent collections with the wet-vacuum system utilized minor headset movement over the sample. That is, the head of the M-Vac® was localized around the area where the sample was spotted for each substrate.

Collection of Blood Samples with the M-Vac® System

Prior to sample collection, the M-Vac® was set-up as previously described. The buffer was placed into the designated chamber and the tubing was attached (Figure 2). Collection bottles and the M-Vac® headset were cleaned according to Figures 1 and 3. For each collection, with the vacuum switch in the off position, the headset of the wet-vacuum system was placed at a 90° angle to the surface of the substrate over the location where sample was spotted. Simultaneously, the vacuum switch was turned on and the buffer switch located on the headset was

pushed to the on position. By steadily holding the headset on the surface of the substrate, as determined through previous experimentation, the headset was localized over the location of the sample with small movement around this area using constant pressure. A total volume of approximately 100 mL of buffer was collected for each sample. When 100 mL of buffer was collected, the buffer switch was turned off while the vacuum remained on and the headset continued to be in contact with the substrate for approximately 5 seconds to vacuum any residual buffer. The vacuum was then switched off and the collection bottle was removed and covered.

Following this, the solutions within the collection bottles were concentrated using the vacuum filtration protocol described above [31]. Like the other collection methods, a substrate control was collected on each type of prepared substrate. In addition, a cleaning blank using a sterile cotton swab moistened with DI H_2O was collected for the vacuum filtration procedure by rubbing the swab, with spinning motion, on the glassware. The swab was focused around the areas of the glassware where DNA may have come into contact. A single cleaning blank was performed for each collection set run through the vacuum filtration procedure.

The filters, substrate controls, and cleaning blanks from the vacuum filtration technique were allowed to dry overnight.

DNA Extraction

All samples were extracted using the QIAamp® Investigator extraction protocol (Qiagen, Valencia, CA). This procedure was performed according to the manufacturer's recommendations outlined in the *Isolation of Total DNA from Surface and Buccal Swabs* [36]. First, whole blood extractions using 100 µL of each dilution (Table 1) were performed. When extracting swabs, the cotton portions of all swabs were cut using sterile scalpels and placed into 2 mL microcentrifuge tubes. Prior to placing the tape into a microcentrifuge tube, each 1 cm flap used to hold the tape during collection was removed and discarded. The tape was then cut into small pieces with sterile scissors and placed in the 2 mL microcentrifuge tubes. The filters used during the filtration procedure, which followed wet-vacuum collection, were first cut into two sections representing 25% and 75% of the filter. As stated previously, only 75 µL of sample was collected for this research while another 25 µL of sample was collected for a separate presumptive testing study [33]. When using the M-Vac® system to collect, a full 100 µL needed to be collected at the same time. Therefore, after drying overnight, the filters were cut into pieces representing 25% and 75% of the total sample. It was assumed that, in the concentration of the sample collected during the vacuum filtration method, the sample would be evenly distributed across the filter; thus, 25% of the filter would have approximately 25 µL of the sample and 75% of the filter would have approximately 75 µL of the sample. Only the 75%

filter piece was used in this portion of the research. Each filter was then cut into small pieces and placed into a 2 mL microcentrifuge tube.

After transferring the substrates to the tubes, 20 µL of Proteinase K and 600 µL of Buffer ATL were added to each tube and incubated at 56°C for 1 hour. During this time, the tubes were vortexed approximately every 10 minutes. Following incubation, 600 µL of Buffer AL was added. Before this addition, 1 µL carrier RNA was added to every 600 µL of Buffer AL, as per the manufacturer's recommendations [36]. The tubes were then incubated at 70°C for 10 minutes. Next, 300 µL of pure ethanol was added to each sample, followed by a 'piggyback' spin to collect all lysate from the substrate. Each lysate was then placed into a QIAamp® MinElute column and centrifuged at 6000 x g for 1 minute. Each sample was washed with 500 µL of Buffer AW1, 700 µL of Buffer AW2, and 700 µL of pure ethanol. Following these wash steps, a new collection tube was inserted under the columns and each tube was centrifuged at full speed for 3 minutes. After centrifugation, the columns were placed into 1.5 mL microcentrifuge tubes and were allowed to sit at room temperature, with the lids open, for 10 minutes. Subsequently, 25 µL of Buffer ATE was added to the center of the membrane on the column and incubated at room temperature for 5 minutes. Each tube was then centrifuged at full speed for 1 minute and the end volume for each sample was assumed to be 20 µL.

DNA Quantification

DNA quantification was performed on all samples using the Quantifiler® Duo Quantification Kit (Applied Biosystems, Foster City, CA). The Master Mix containing both Duo Primer Mix and Duo Reaction Mix was prepared according to the manufacturer's recommendations [21]. When preparing the plate, each sample volume totaled 25 µL, including 23 µL of the Master Mix and 2 µL of the extracted DNA sample.

During amplification, the quantity of DNA within each sample was detected using the 7500 Detection System (Applied Biosystems, Foster City, CA) and the results were then analyzed using a publicly available Microsoft Excel template [38].

STR Profiling

Some of the samples, including the replicate from each collection set with the highest yield and the substrate controls, were subjected to STR analysis. Amplification was performed using the PowerPlex® 16 HS System (Promega, Madison, WI) and a target of either 0.7 ng or 10 µL of extract. Capillary electrophoresis was performed using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Further analysis was performed using the GeneMapper® ID-X Software (Applied Biosystems, Foster City, CA), using an analytical threshold of 50 RFU.

Statistical Analysis

Statistical analysis was performed using Microsoft® Excel® for Mac 2011 (Microsoft, Redmond, WA) and the StatPlus®:mac LE statistical analysis program (AnalystSoft Inc., Vancouver, BC, Canada).

Visual Outline of Materials and Methods

Figure 4 represents a consolidated outline of the methods and procedures performed for this study.

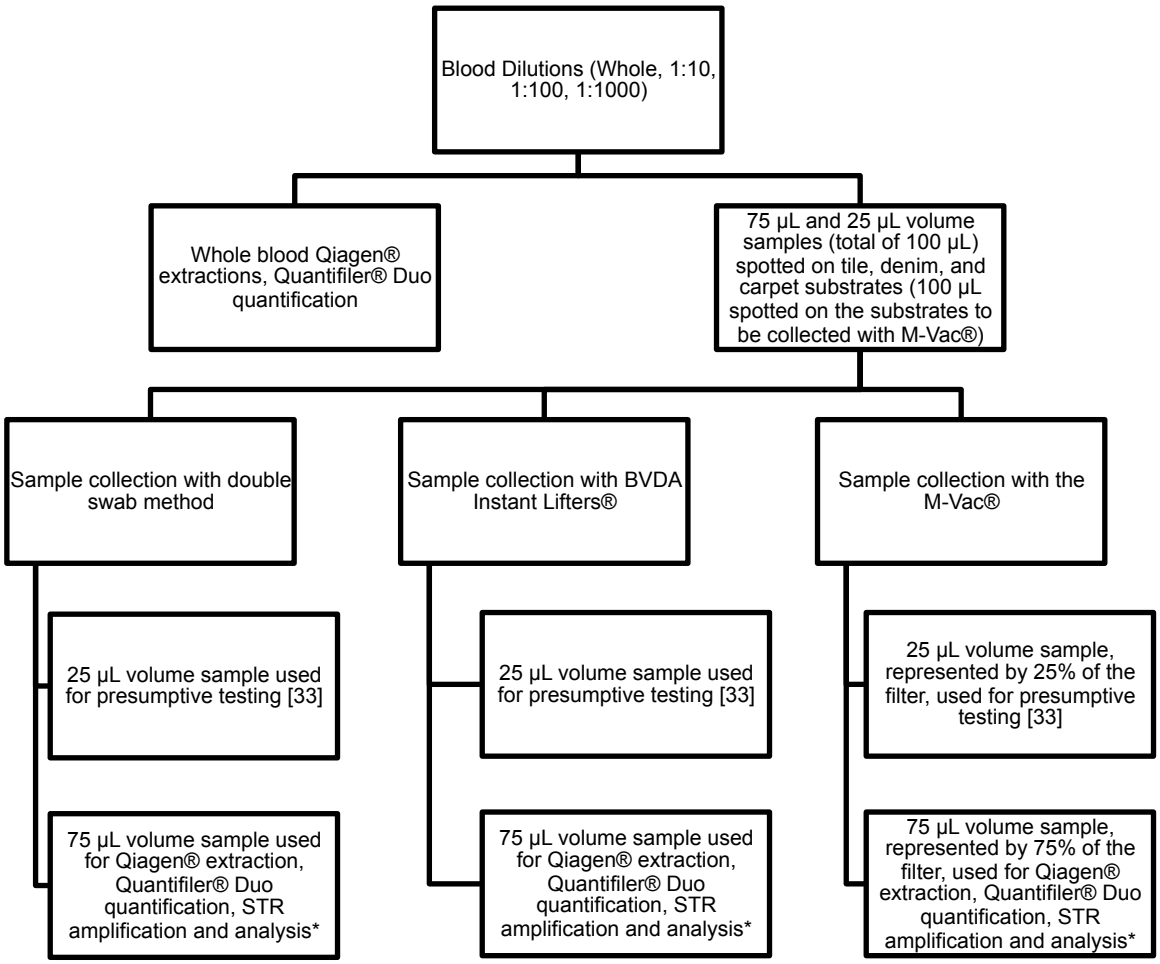

Figure 4. *Outline of methods utilized from sample/substrate preparation to sample collection and analysis. *Only one of the three replicates was used for STR analysis.*

Results and Discussion

Raw Data and Percent Recovery

As previously described, each sample collected was quantified via qPCR using the Quantifiler® Duo Quantification Kit and the 7500 Detection System. Therefore, the data acquired from this process represented the signal at which the samples were measured (the C_T value) and the concentration of DNA (in ng/µL) measured at this specific signal [39]. The most fundamental comparison that can be made with the samples collected from each biological collection technique is a comparison between the amounts of DNA extracted.

To compare these values, the average concentration for each sample was determined. In addition, the average concentrations of DNA detected for the whole blood extractions were also calculated. It is important to note that the average concentrations for the whole blood extractions were first calculated and then multiplied by ¾ (i.e. 0.75). This was done because the whole blood extractions performed used 100 µL of the sample dilutions instead of 75 µL. By multiplying the results given for the whole blood extractions by ¾ (i.e. 0.75), this represents the average concentration of the whole blood extractions as if 75 µL was extracted. This then allows a direct comparison of the results observed for the different biological collection techniques to the whole blood extractions to be performed. Tables 3-5 show the average concentrations detected for each substrate, target volume of sample, and collection method.

Table 3. *Average concentrations of blood (0.075 – 75 µL) with 2SD collected from tile using various collection methods (in ng/µL).*

Collection Method	75 µL Blood	7.5 µL Blood	0.75 µL Blood	0.075 µL Blood
Whole Blood	52 (± 15)	6 (± 7)	0.50 (± 0.08)	0.04 (± 0.03)
Double Swab	75 (± 14)	3 (± 3)	0.16 (± 0.08)	0.01 (± 0.01)
Tape (BVDA Instant Lifters®)	50 (± 28)	1 (± 1)	0.1 (± 0.1)	0.02 (± 0.02)
Wet-Vacuum Collection (M-Vac®)	66 (± 7)	3 (± 2)	0.2 (± 0.1)	0.01 (± 0.02)

Table 4. *Average concentrations of blood (0.075 – 75 µL) with 2SD collected from denim using various collection methods (in ng/µL).*

Collection Method	75 µL Blood	7.5 µL Blood	0.75 µL Blood	0.075 µL Blood
Whole Blood	52 (± 15)	6 (± 7)	0.50 (± 0.08)	0.04 (± 0.03)
Double Swab	9 (± 1)	0.5 (± 0.4)	0.01 (± 0.01)	0.001 (± 0.004)
Tape (BVDA Instant Lifters®)	3 (± 3)	2 (± 1)	0.1 (± 0.2)	0.004 (± 0.004)
Wet-Vacuum Collection (M-Vac®)	64 (± 3)	4.8 (± 0.2)	0.16 (± 0.04)	0.02 (± 0.04)

Table 5. *Average concentrations of blood (0.075 – 75 µL) with 2SD collected from carpet using various collection methods (in ng/µL).*

Collection Method	75 µL Blood	7.5 µL Blood	0.75 µL Blood	0.075 µL Blood
Whole Blood	52 (± 15)	6 (± 7)	0.50 (± 0.08)	0.04 (± 0.03)
Double Swab	27 (± 9)	1 (± 2)	0.010 (± 0.006)	0.001 (± 0.003)
Tape (BVDA Instant Lifters®)	9 (± 2)	0.3 (± 0.2)	0.1 (± 0.2)	0.001 (± 0.002)
Wet-Vacuum Collection (M-Vac®)	36 (± 12)	0.6 (± 0.5)	0.08 (± 0.08)	0.03 (± 0.02)

When reviewing the average concentrations of DNA collected with each method, a general trend emerges. For example, for the 0.75 µL blood samples, the amount of DNA obtained on all substrates utilizing the M-Vac® was greater than what was collected using the double swab method. However, in order to see more clearly how the concentrations of DNA detected for each method compare to each other, it is imperative these concentrations be directly compared back to the concentrations detected for the whole blood extractions. This was accomplished through the calculation of the percent recovery of DNA for each sample using each collection technique.

For the whole blood extractions, 75 µL of whole blood yielded 52 ± 15 ng/µL, 7.5 µL of whole blood yielded 6 ± 7 ng/µL, 0.75 µL of whole blood yielded 0.50 ± 0.08 ng/µL, and 0.075 µL of whole blood yielded 0.04 ± 0.03 ng/µL. The percent recovery was calculated by taking the average concentration of DNA collected for each sample divided by the average concentration of DNA detected

for the whole blood extractions. While this approach allows for a more accurate comparison, it was also important to determine the error with respect to the collection of samples with a specific method and the whole blood extractions along with their associated standard deviations. The error of percent DNA recovery was calculated using the theory of the propagation of random error [40-42]:

$$\boldsymbol{\sigma_y} = \boldsymbol{y}\sqrt{\left(\left(\frac{\sigma_a}{a}\right)^2 + \left(\frac{\sigma_b}{b}\right)^2\right)} \qquad \text{(Equation 1)}$$

where *y* represents the percent DNA recovery, *a* is the average DNA concentration collected from a specific collection technique at a specific DNA target amount, σ_a is the standard deviation of *a*, *b* is the average DNA concentration from the whole blood extractions at a specific DNA target amount, and σ_b is the standard deviation of *b*. Thus, σ_y represents the error of the percent DNA recovery.

Overall, it is observed that the average percent DNA recovery varied for each type of biological collection technique depending on both the type of substrate and the sample volume placed on the substrate. Figures 5-7 represent the average percent DNA recovery from each substrate and its associated error multiplied by two for each target volume of blood for the three collection methods utilized.

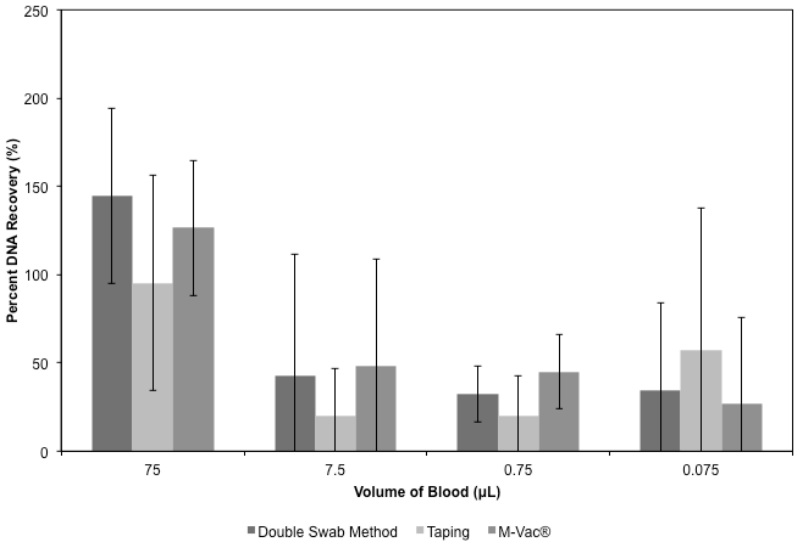

Figure 5. *Percent DNA recovery of blood (0.075 – 75 µL) using various collection methods on tile with error bars representing 2SD calculated using the theory of the propagation of random error.*

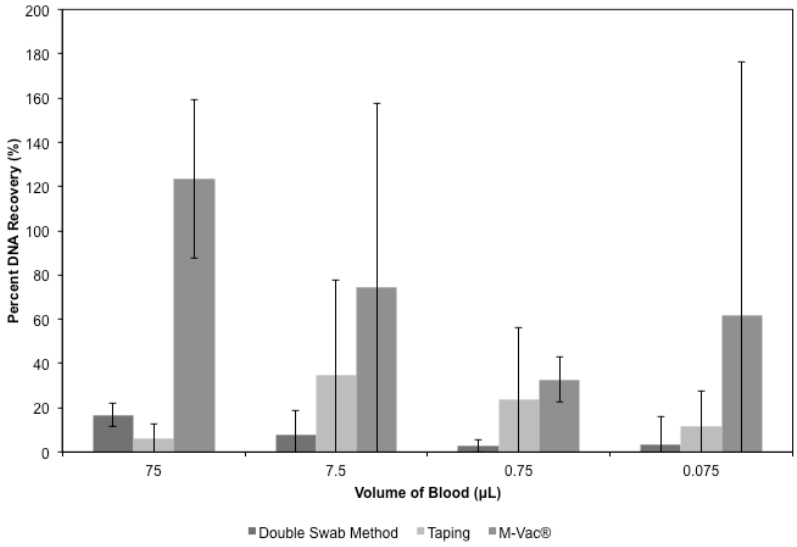

Figure 6. *Percent DNA recovery of blood (0.075 – 75 µL) using various collection methods on denim with error bars representing 2SD calculated using the theory of the propagation of random error.*

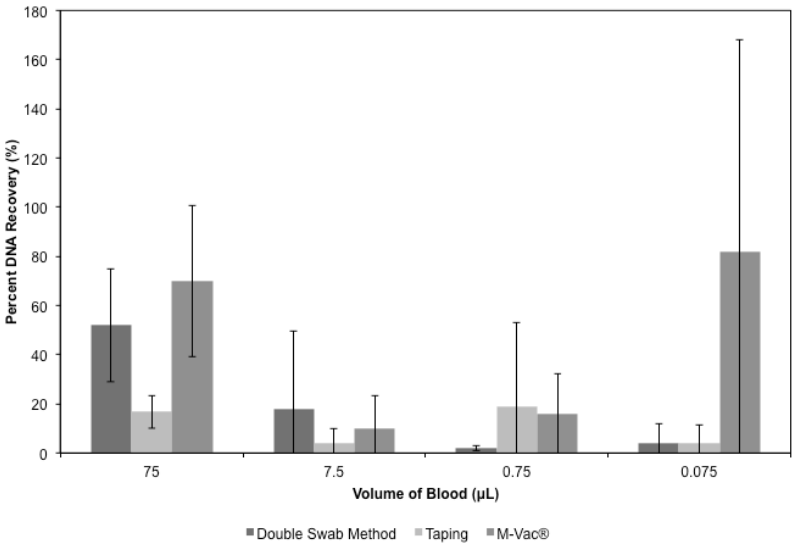

Figure 7. *Percent DNA recovery of blood (0.075 – 75 µL) using various collection methods on carpet with error bars representing 2SD calculated using the theory of the propagation of random error.*

On tile, the wet-vacuum system and the double swab method recovered more DNA than that of the taping method for target volumes of 0.75 – 75 µL. For a target volume of 75 µL the double swab method was able to recover similar levels of DNA when compared to wet-vacuum collection, with a total percent recovery of 144 ± 50 % and 127 ± 38 % respectively. At this target volume, tape recovered 95 ± 61 % DNA. For a target volume of 7.5 µL on tile, the wet-vacuum was able to recover 48 ± 60 % DNA while the double swab method recovered similar levels, showing 42 ± 69 % DNA recovery. Taping recovered slightly less with 20 ± 27 % DNA recovery. This trend was also observed for a target volume of 0.75 µL in which the wet-vacuum system recovered 45 ± 21 % DNA. The double swab method recovered similar levels with 33 ± 16 % DNA and the taping method recovered slightly less at 20 ± 23 % DNA. Interestingly, on tile, with a

volume of 0.075 µL, the taping method resulted in a slightly higher DNA percent recovery, with 57 ± 80 % DNA, as compared to the double swab method and the M-Vac®, that recovered 34 ± 50 % DNA and 27 ± 49 % DNA, respectively.

Unlike tile, the collection method did seem to significantly affect the ability to collect the biological specimen when denim was the substrate. While there were many cases of the various collection methods on the non-porous substrate resulting in similar yields, this was not observed on the sample collections performed on denim. For each target volume of blood, the use of the wet-vacuum system recovered a higher percentage of DNA than the double swab and taping methods. Further, the differences between the collection techniques in the percent DNA recovery were higher, especially when comparing the M-Vac® to the other collection techniques. For the wet-vacuum sample collection of 75 µL on denim, there was a 124 ± 36 % DNA recovery. This was significantly more than the DNA recovery obtained when utilizing the double swab method and taping, in which only 17 ± 5 % and 6 ± 7 % DNA was recovered. For a target volume of 7.5 µL, 74 ± 83 % DNA was recovered from the denim using the wet-vacuum, 8 ± 11 % DNA was recovered using the double swab method, and 35 ± 43 % DNA was recovered using the taping method. For a target volume of 0.75 µL, the wet-vacuum system recovered 33 ± 10 % DNA while taping recovered similar levels with a recovery of 24 ± 32 % DNA and the double swab method fell short of this with a percent DNA recovery of 3 ± 3 %. The M-Vac® was, again, able to recover more DNA than the other two methods with a target volume of

0.075 µL with 62 ± 115 % DNA recovery as compared to 4 ± 13 % DNA recovery with the use of the double swab method and 12 ± 16 % DNA recovery using the taping method.

Although it is clear that the M-Vac® collected more DNA from the denim for all target volumes of blood, it is also important to note that taping recovered more DNA at the lower volumes (0.075 – 7.5 µL) than the double swab method. This may become a factor to consider when making recommendations or developing protocols on which collection technique to use in the field.

When reviewing the results obtained solely from tile and denim, a relationship was observed between substrate type and the ability of the collection technique to recover the DNA. For tile, the differences between DNA recoveries using various collection techniques were smaller than the differences observed when denim was the substrate. On a non-porous surface, it is reasonable that the percent DNA recovery between each method was generally closer due to the fact that the sample did not wick into the substrate. Although the samples were allowed to dry, it was observed that the blood seemed to sit on the top surface of the substrate, essentially making each method perform similarly to each other. This was not the case for the denim substrate. Therefore, it is hypothesized that the use of the wet-vacuum system's pressurized output of solution, along with subsequent vacuuming, agitated the dried sample on the denim much more than the disruption that was caused by the double swab technique and the taping

method. This extra agitation may have made it easier for the M-Vac® to collect more of the sample overall and, thus, have higher DNA percent recoveries.

The third and final substrate tested was carpet, also a porous substrate. It might have been assumed that the different biological collection techniques would have behaved much like the samples collected on denim due to reasons stated above. However, this was not the case. For the samples collected on carpet, each collection technique had at least one target volume of blood in which it performed best. For a target volume of 75 µL, the wet-vacuum system recovered the most DNA with a recovery of 70 ± 31 % DNA. At this target volume the double swab method recovered 52 ± 23 % DNA and the taping method recovered 17 ± 7 % DNA. For a target volume of 7.5 µL, the double swab technique recovered the most DNA with a recovery of 18 ± 32 % DNA while the wet-vacuum recovered 10 ± 13 % and taping recovered 4 ± 6 %. For a target volume of 0.75 µL, the taping method recovered a higher percent of DNA with 19 ± 35 % DNA recovered. The wet-vacuum system collected the second highest percentage of DNA at this target volume with 16 ± 17 % DNA recovery and the double swab method recovered 2 ± 1 % DNA. For a target volume of 0.075 µL, the wet-vacuum outperformed both the double swab method and taping with a percent DNA recovery of 82 ± 86 %. The double swab method and taping only recovered 4 ± 8 % and 4 ± 8 % DNA, respectively.

While the explanation for the M-Vac® recovering more DNA for 75 µL and 0.075 µL of blood than the double swab method and taping may be the same for

what was seen for the denim - due to the output of solution and subsequent vacuuming - it seems that the nature of the type of substrate becomes even more important with the results obtained from the carpet. While these results will ultimately be used to propose a suggestion as to which biological collection technique to use, more investigation on the effect specific substrates have on collection is required.

It is also important to note that there were times where the percent DNA recovery was calculated to be greater than 100%. This may be attributed to the fact that the sample collections were compared to an average concentration based on a range of DNA concentration values observed for the whole blood extractions. Overall, there was variability within the whole blood extractions, as observed by the standard deviations. Therefore, it is inevitable that there may be instances in which the percent DNA recovery observed would be above 100%. It is also hypothesized that, when collecting from the denim and carpet, the percent DNA recoveries above 100% could have been attributed to possible interference DNA already present on the substrates.

Internal Positive Control

While it is important to assess and analyze the raw data for comparison of these three collection techniques, it is just as imperative to assess whether there was any inhibition during the qPCR process, which can be caused by a number of factors [25, 27]. In order to pinpoint whether there were difficulties during

qPCR due to inhibitors for each collection performed, the fluorescence measured at every cycle of qPCR and the C_T values of the internal positive control (IPC) were examined. Figures 8-10 demonstrate the change in fluorescence of the IPC with respect to the cycle number of the original qPCR reactions. In addition, the average C_T values are also presented with two standard deviations. These figures include all of the IPC data from each quantification performed for the samples of blood collected from each substrate using the various collection techniques.

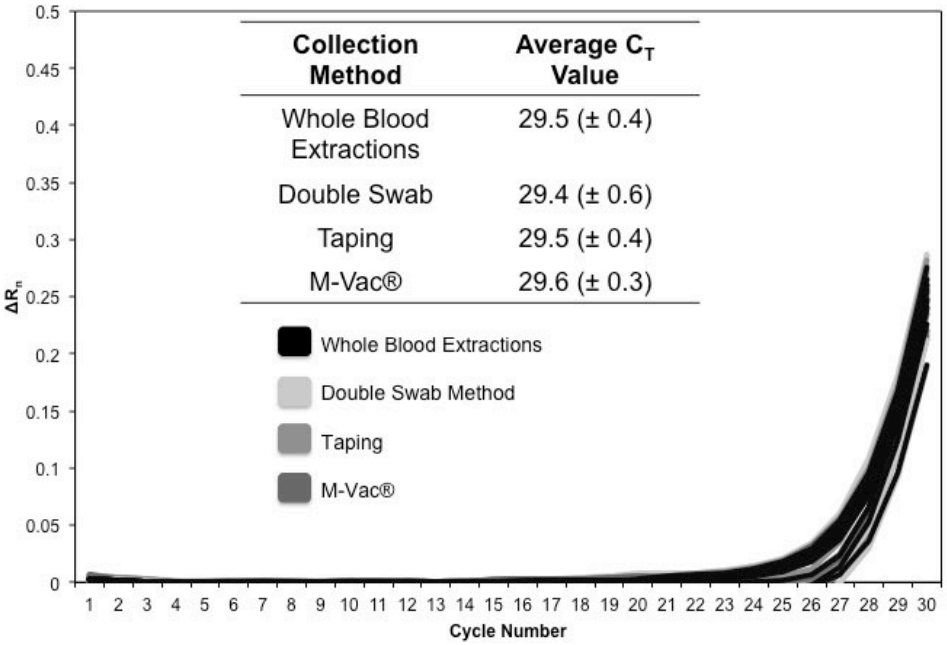

Figure 8. *IPC analysis of each collection method from tile with average C_T values (± 2SD).*

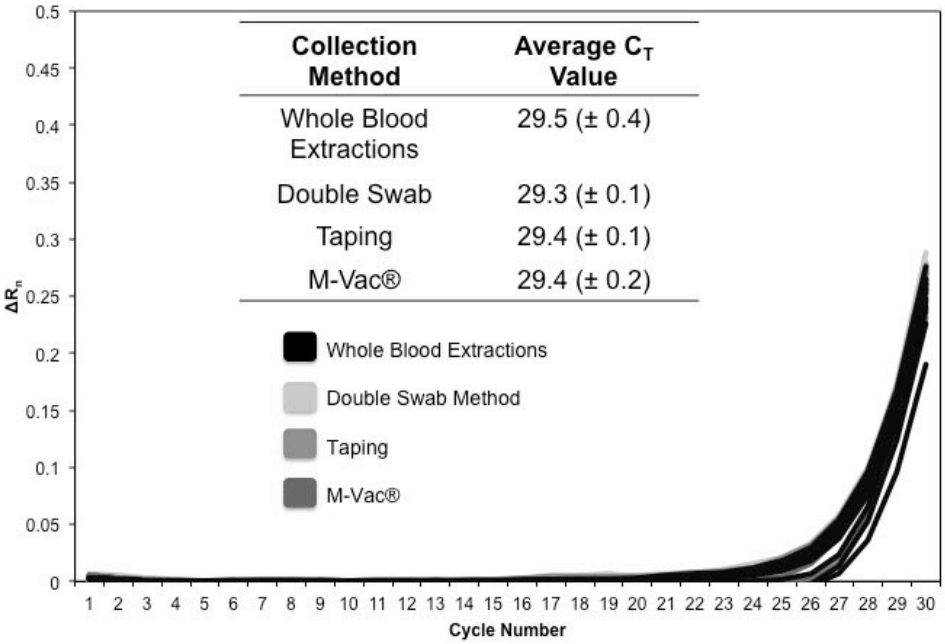

Figure 9. *IPC analysis of each collection method from denim with average C_T values (± 2SD).*

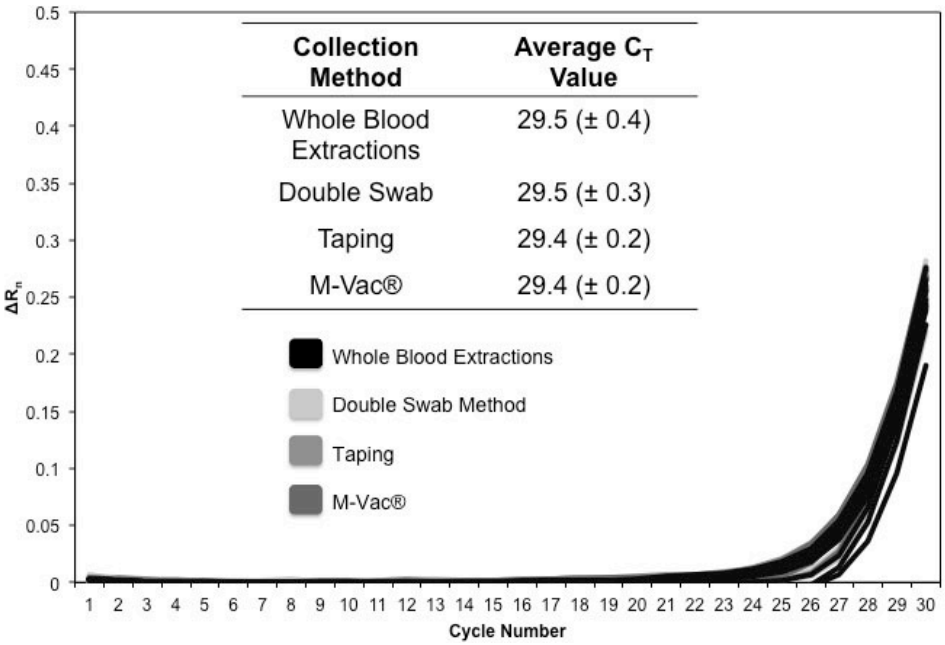

Figure 10. *IPC analysis of each collection method from carpet with average C_T values (± 2SD).*

From this data, it was determined that the average C_T values for the IPCs for the whole blood was 29.5 ± 0.4. In comparison, the C_T values for all collections performed were similar, with the highest average C_T value being 29.6 ± 0.3 detected from the collections performed using the M-Vac® on tile and the lowest average C_T value being 29.3 ± 0.1 detected from the collections performed using the double swab method on denim. All other average C_T values from the IPCs were between these values. The consistency seen within the data for the IPC for each collection method suggests there was no significant inhibition during amplification and quantification for any of the substrates.

Minimal Distinguishable Signal

The minimum distinguishable signal (MDS) represents the minimum analytical signal that a specific protocol can detect with reasonable certainty [43] and is based on the average signals of the blanks (i.e. the substrate controls). Ultimately, this allows an analyst to determine the minimum required signal to distinguish true detection from noise.

The MDS for qPCR is stated in terms of the C_T (cycle threshold) – i.e. the cycle at which the fluorescent signal crosses a specified threshold [39]. Therefore, in the case of qPCR, the MDS was calculated by taking the average C_T value from the blanks/substrate controls run for the whole blood extractions and each sample collection, designated as the minimum blank signal (MBS), and subtracting three standard deviations:

$$\boldsymbol{MDS = MBS - 3s_{bl}} \qquad \text{(Equation 2)}$$

For some of the collections performed, there was no detection of DNA and, therefore, no quantification data for the blanks. The MBS for these blanks was determined by taking into consideration that the highest set C_T value for the Quantifiler® Duo quantification procedure is 40. Because there can be no detection past this point, the final C_T value for the blanks with no detectable DNA was considered to have an MBS of 40 C_T. This poses a problem in calculating the MDS of these blanks due to the need to take into consideration the inherent error of the blank signals. To determine the error associated with a C_T value of 40, a regression that predicts the error of the qPCR process at specific C_T values was created utilizing qPCR standard curves [44]. This regression can be seen in Figure 11 and represents the estimated error with respect to signal.

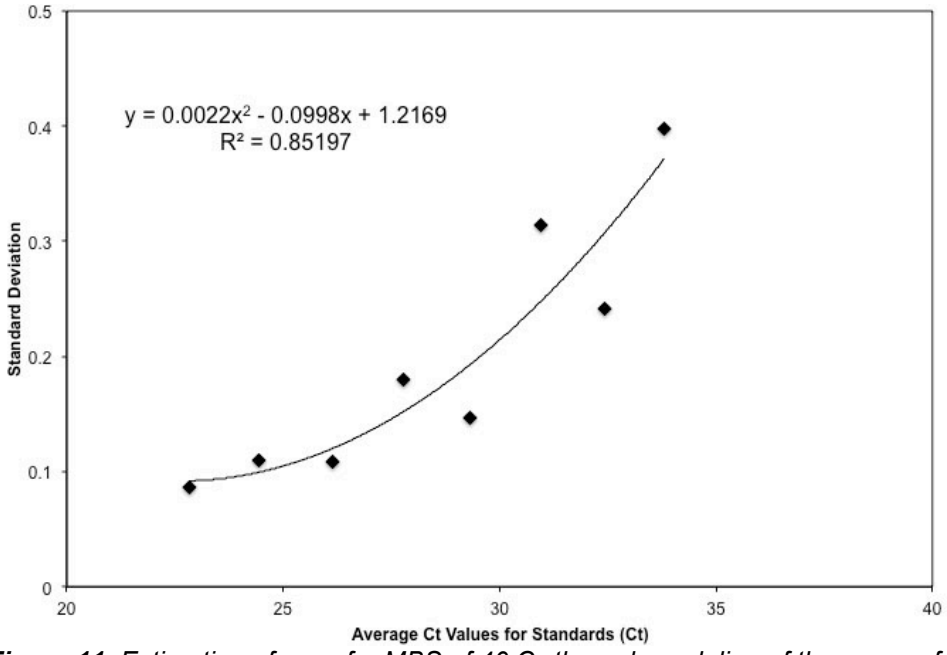

Figure 11. *Estimation of error for MBS of 40 C_T through modeling of the errors of the real-time PCR standards with respect to their average C_T values.*

Qualitatively, it is observed that the relationship between the standard deviation and average signal - i.e. the C_T value - was not linear and, therefore, a polynomial regression was utilized. With this, the error associated with an MBS of 40 C_T was calculated by inputting 40 into the polynomial equation (Figure 11) and was estimated to be 0.7449. Thus, the MDS was determined to be 37.8 C_T (Equation 2) for the associated blanks.

As a result, 37.8 C_T was the MDS for the whole blood extractions and the following collections: double swab method on tile, double swab method on denim, double swab method on carpet, taping on tile, and the M-Vac® on tile. The MDSs calculated for the taping method on denim and carpet were found to be lower than the blanks with an MBS of 40 C_T. DNA signal was observed for 2 of 4 denim blanks and 1 of 4 carpet blanks. Therefore, the MDS for taping was found to be 36.3 C_T on denim and 36.0 C_T on carpet. This suggests that the minimum signal that can be accurately determined and is separated out from the baseline noise with higher certainty is lower than those collections where the MBS was 40 C_T. This proved to be even lower for the collections performed utilizing the wet-vacuum system on denim and carpet. For these collections, DNA signal was observed for 3 of 4 denim blanks and 4 of 4 carpet blanks. The MDS for the wet-vacuum system was 29.6 C_T on denim and 33.4 C_T on carpet. A summary of these results is presented in Table 6.

Table 6. *Minimum base signals and their respective standard deviations used to calculate minimum distinguishable signals for whole blood extractions and each collection performed.*

Collection Performed	Substrate	Minimum Base Signal (MBS) (C_T)	Standard Deviation	Minimum Distinguishable Signal (MDS) (C_T)
Whole Blood Extractions	N/A	40	0.74	37.8
Double Swab Method	Tile	40	0.74	37.8
	Denim	40	0.74	37.8
	Carpet	40	0.74	37.8
Taping	Tile	40	0.74	37.8
	Denim	39.24	0.99	36.3
	Carpet	39.43	1.13	36.0
Wet-Vacuum System (M-Vac®)	Tile	40	0.74	37.8
	Denim	36.55	2.33	29.6
	Carpet	34.55	0.38	33.4

These MDSs are important when evaluating each of the collection techniques. It must be emphasized that the lower the MDS, the higher the concentration of DNA needed to distinguish between signal and background. That is, a procedure with a lower MDS must essentially collect more DNA in order to overcome the baseline noise/interference to detect the sample. While the collections performed on tile for each method were the same in terms of MDS, when using the taping technique and the M-Vac® on denim and carpet, the lower MDSs must be taken into consideration when determining their optimal use in the field. For example, if there is a situation in which there is a dilute stain on carpet,

one must take into account the fact that if taping or a wet-vacuum system is to be used, the baseline noise (i.e. background DNA) may be higher. This then makes the MDS lower in order to detect the DNA concentration with greater certainty.

Limit of Detection

The limit of detection (LOD) represents another figure of merit that can help compare between collection techniques and is described as the lowest concentration at which an analyte can be reliably detected. That is, the LOD represents the lowest concentration that can be accurately distinguished from background noise [40].

In order to calculate the LOD, a comparison of the volume of whole blood collected (the target volume) to the average signal (C_T) detected for each collection method and substrate was made and a logarithmic regression ensued. This was performed to determine the slope, y-intercept, and their respective errors through regression analysis. These values are then integrated into the LOD equation (Equation 3-5). Figures 12-15 show the curves created for each collection performed and their respective regression equations.

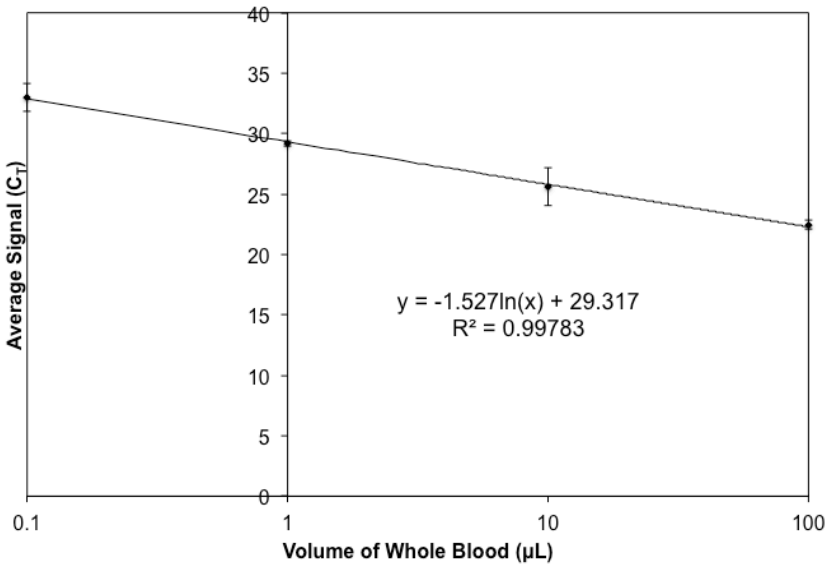

Figure 12. *Logarithmic regression of the average C_T values with respect to volume of blood (µL) for use in the calculation of the LOD for the whole blood extractions.*

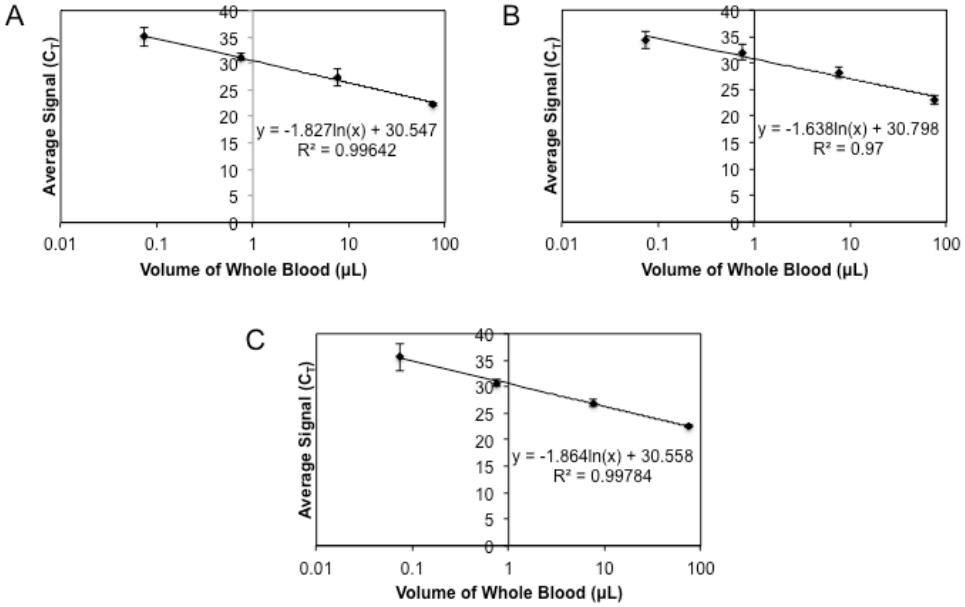

Figure 13. *Logarithmic regression of the average C_T values with respect to volume of blood (µL) for use in the calculation of the LOD on tile for (A) the double swab method, (B) taping, (C) and the M-Vac®.*

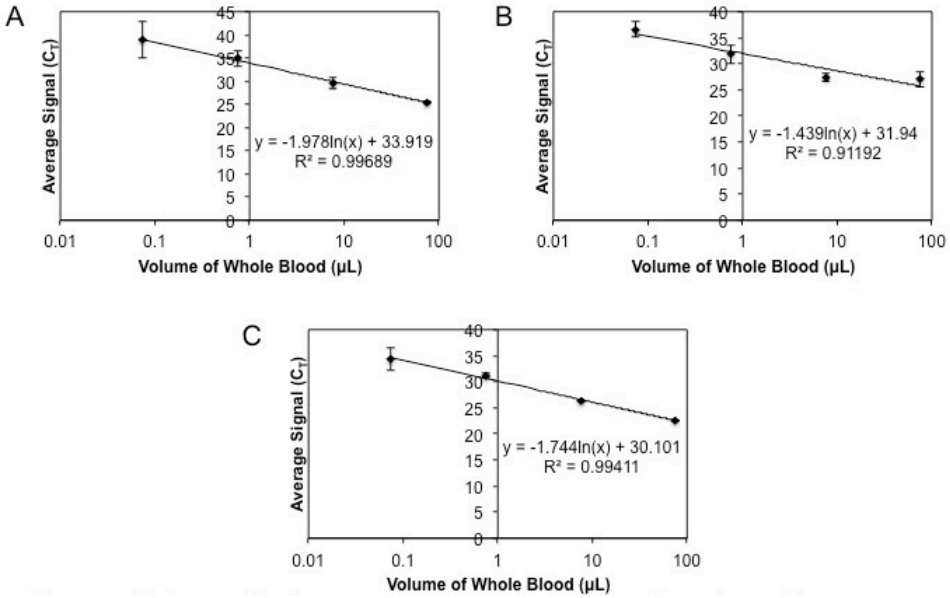

Figure 14. *Logarithmic regression of the average C_T values with respect to volume of blood (µL) for use in the calculation of the LOD on denim for (A) the double swab method, (B) taping, (C) and the M-Vac®.*

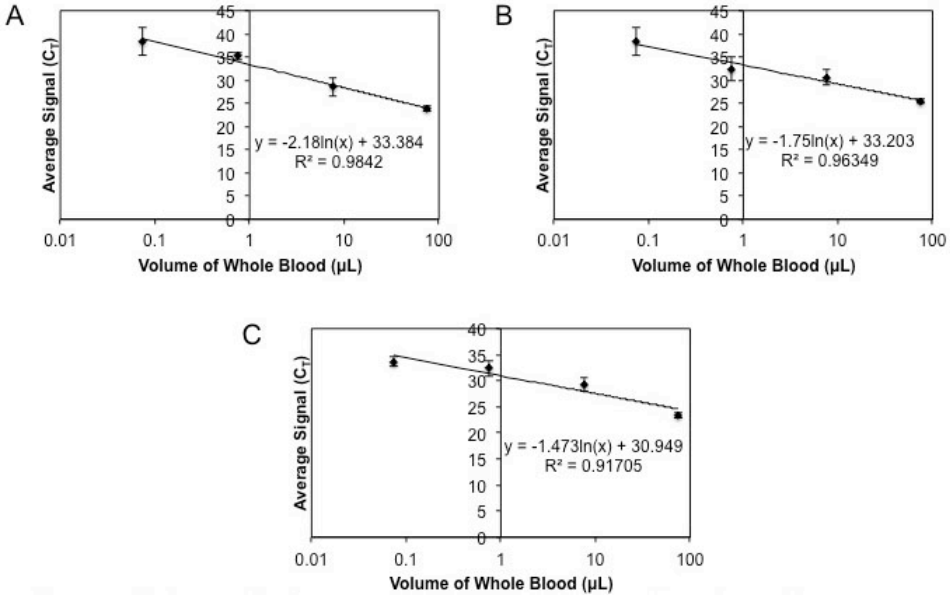

Figure 15. *Logarithmic regression of the average C_T values with respect to volume of blood (µL) for use in the calculation of the LOD on carpet for (A) the double swab method, (B) taping, (C) and the M-Vac®.*

The LOD can be computed by determining a base volume (BV) through the use of the MBS and adding three times the error found utilizing the theory of the propagation of error, as suggested by Winefordner [45]. As stated earlier, the slope and y-intercept were determined through the use of a logarithmic regression that is in the form:

$$\boldsymbol{y = m \ln x + b} \qquad \text{(Equation 3)}$$

where *y* represents the MBS, *m* represents the slope, *x* represents the base volume (BV) in which the final LOD will be calculated from, and *b* represents the y-intercept. When rearranged, the BV was determined for each sample collection:

$$\boldsymbol{x = e^{\frac{(y-b)}{m}}} \qquad \text{(Equation 4)}$$

After calculating the BV, which is denoted '*x*' in Equation 3 and 4, the theory of the propagation of error assisted in acquiring the associated deviation of the BV. Ultimately, the equation for the BV error was employed as follows:

$$\boldsymbol{\sigma_x = \frac{x}{|m|}\sqrt{\left(\sigma_y{}^2 + \sigma_b{}^2 + \left(\frac{b-y}{m}\right)^2 \sigma_m{}^2\right)}} \qquad \text{(Equation 5)}$$

where *x* represents the BV as determined through Equation 4, *m* represents the slope, *b* represents the y-intercept, *y* represents the MBS, σ_y represents the standard deviation associated with the MBS, σ_b represents the standard deviation associated with the y-intercept, and σ_m represents the standard deviation associated with the slope. Table 7 demonstrates the approximated BV and LOD for each collection method with respect to the type of substrate.

Table 7. *Base volume and limit of detection calculated for whole blood extractions and each collection performed.*

Collection Performed	Substrate	Base Volume (BV) (nL); Calculated Using Equation 4	Limit of Detection (LOD) (nL); Calculated Using Equation 4 and 5
Whole Blood Extractions	N/A	0.9	2.4
Double Swab Method	Tile	6	14
	Denim	50	100
	Carpet	50	120
Taping	Tile	4	13
	Denim	6	33
	Carpet	30	100
Wet-Vacuum System (M-Vac®)	Tile	6	15
	Denim	20	130
	Carpet	90	300

The LOD determined for the whole blood extractions was 2.4 nL. That is, when extracting whole bloods using Qiagen extraction and performing qPCR, only 2.4 nL of whole blood is required to obtain reliable detection and supports the common view that DNA profiling is a powerful tool for forensic purposes. When collection is required, the LODs increase as expected, and is presumed to be due to the loss of sample during collection and extraction.

When collecting on tile, the wet-vacuum system, the double swab method, and taping all resulted in similar LODs of 15, 14, and 13 nL, respectively.

In the case of sample collection on denim, the taping method resulted in the lowest LOD of 33 nL. The LODs calculated for the double swab method, 100

nL, and the M-Vac®, 130 nL, were significantly larger when collecting from this substrate, suggesting the double swab or wet-vacuum methods would have to extract much more DNA from the denim substrate in order to detect the target DNA over the interference with any certainty. A possible reason as to why the double swab method and the wet-vacuum resulted in higher LODs on the denim substrate could be due to the simultaneous rehydration of the sample as well as the actual substrate itself. This liquid saturation does not occur with the taping method and may have resulted in the double swab method and the M-Vac® collecting DNA material from both the sample and the underlying DNA already present on the substrate.

This trend was also observed for the collections performed on carpet. The taping method resulted in an LOD of 100 nL, the double swab method resulted in an LOD of 120 nL, and the M-Vac® resulted in an LOD of 300 nL. While the LOD for taping and the double swab method were similar, the double swab method may have resulted in a slight elevation in LOD due to the same rehydration reasons as stated for the denim substrate. Alternatively, the sample collections performed with the wet-vacuum system resulted in a significant increase in the LOD over the other two methods. It is hypothesized that the pressurized output of buffer from the M-Vac® could have resulted in higher levels of background noise or interference because it may have been collecting nuclear material already present, and from a deeper level within the carpet, and not solely from the surface of the carpet where the DNA was spotted. In contrast, the double swab

method and taping allowed for a more controlled depth of sampling, leading to lower LODs. This suggests that, when sampling carpet, the taping and double swab method may be able to reliably detect smaller amounts of the sample of interest, leading to increased specificity during collection. This is particularly true if the stain of interest is visible and on the surface of the porous substrate. It is important to note that the LODs found for all collection techniques increased when going from tile to denim and increased even more when going from denim to carpet due to the existence of interfering nuclear material already present on the substrates. All of the LODs were higher than the LOD determined for the whole blood extractions.

STR Profiling: Minimum Number of Contributors and Average Peak Height

To further examine the three collection techniques, a number of STR profiles were obtained. For each collection performed as well as the whole blood extractions, only the replicate with the highest concentration of DNA detected through quantification was subjected to amplification using the PowerPlex® 16 HS System and capillary electrophoresis. The profiles were analyzed utilizing the GeneMapper® ID-X Software with an analytical threshold of 50 RFU. For each sample profile, the various types of artifacts, including stutter, minus A, and bleed through [39], were removed.

The first aspect of the profiles that was determined was the minimum number of contributors (Table 8). This number was approximated by counting the

number of alleles at a given locus, dividing by two, and rounding up. The minimum number of contributors for the substrate controls performed with every collection set is also presented (Table 9).

Table 8. *Minimum number of contributors determined from STR profiles examined from samples of 0.075 – 75 µL blood collected using various collection methods.*

Collection Performed	Substrate	75 µL of Blood Spotted	7.5 µL of Blood Spotted	0.75 µL of Blood Spotted	0.075 µL of Blood Spotted
Whole Blood Extractions	N/A	1	1	1	1
Double Swab Method	Tile	1	1	1	1
	Denim	1	1	1	1
	Carpet	1	1	1	1
Taping	Tile	1	1	1	1
	Denim	1	1	1	2
	Carpet	1	1	1	1
Wet-Vacuum System (M-Vac®)	Tile	1	1	1	1
	Denim	1	1	1	3
	Carpet	1	1	3	4

Table 9. *Minimum number of contributors determined from STR profiles of the substrate controls collected using various collection methods.*

Collection Performed for Substrate Control	Substrate	Minimum Number of Contributors
Double Swab Method	Tile	0
	Denim	0
	Carpet	0
Taping	Tile	0
	Denim	1
	Carpet	0
Wet-Vacuum System (M-Vac®)	Tile	0
	Denim	1
	Carpet	3

The minimum number of contributors was one for most of the samples (Table 8). This is expected since the samples collected were single source. There were, however, a few instances in which there were a minimum number of contributors greater than one. This occurred when denim or carpet was the substrate and when using the taping method or the wet-vacuum system. Therefore, when collecting from porous substrates from high-traffic areas, there may be a chance, for example, when using the M-Vac® on carpet, that the final STR profiles obtained will be complex mixtures. This, in turn, makes the STR profiles harder to interpret and may possibly lead to inconclusive or un-interpretable results. The single source profile obtained for the blood that was used for collection was always present in the profiles.

It was also important to examine the substrate controls run from each collection technique performed. While most profiles from these blanks had no alleles detected, as expected, there were a few instances in which alleles were observed with the denim and carpet substrates (Table 9). This suggests, again, that consideration of which collection technique to use must take into account not only the quantity of DNA, but also whether the substrate is porous and fibrous enough to contain large levels of background DNA. For example, although the wet-vacuum method may be more successful at collecting more DNA, if a bloodstain is visible and on the surface of a high-traffic area (i.e. carpet, chair in a public area, etc.), then taping or swabbing may be the preferred method of collection.

The average peak heights of the alleles detected in the substrate controls were also calculated. Table 10 shows the average peak heights calculated with two standard deviations in relative fluorescence units (RFU).

Table 10. *Average peak height values with 2SD in RFU observed from the STR profiles of the substrate controls.*

Collection Performed for Substrate Control	Substrate	Average Peak Height (RFU)
Double Swab Method	Tile	0 (± 0)
	Denim	0 (± 0)
	Carpet	0 (± 0)
Taping	Tile	0 (± 0)
	Denim	61 (± 9)
	Carpet	0 (± 0)
Wet-Vacuum System (M-Vac®)	Tile	0 (± 0)
	Denim	300 (± 500)
	Carpet	300 (± 400)

This data also indicates the presence of multiple sources of DNA interference from background contributors. This is something that will be important to note, specifically due to the fact that many substrates are not free from interference. That is, background DNA may be present and may complicate DNA interpretation and comparison.

Presumptive Testing

The 0.00025 - 25 µL volume samples reserved for presumptive testing for blood were analyzed using the Kastle-Meyer colorimetric test [33]. This testing was completed in conjunction with the work presented here and included volumes of 0.0025 µL and 0.00025 µL in order to assess the overall sensitivity of

the Kastle-Meyer colorimetric test with respect to the type of collection method and substrate.

In general, for tile, the double swab method tended to yield higher color intensities than that of taping and the wet-vacuum system for all volumes of blood with the exception of 25 µL, in which the M-Vac® resulted in higher intensities than the other two methods. The wet-vacuum results also maintained higher color intensities than those of the taping method at blood volumes of ≥ 0.25 µL.

The overall intensities obtained from the denim substrate using the M-Vac® were higher than taping and swabbing at ≥ 2.5 µL of blood and as well as for 0.025 µL of blood. For volumes ≥ 0.25 µL, the double swab method yielded higher intensities than taping. For blood dilutions of 0.0025 µL and 0.00025 µL, the relative intensities were similar between all collection techniques. For collections performed on carpet, the taping method yielded higher intensities at ≥ 2.5 µL than both the double swab method and the wet-vacuum system. For blood volumes of ≤ 0.25 µL, the relative intensities were comparable between all three collection methods.

Although the intensities calculated assist in representing the overall sensitivity of each method with respect to the presumptive Kastle-Meyer colorimetric test, it is important to note that there were only a few instances at which a positive presumptive test was visually observed for the double swab method and taping that was not visually observed for the M-Vac®. This was only

observed for volumes of blood ≤ 0.025 µL. For blood volumes of ≥ 0.25 µL, all three methods performed similarly. No visual results were indicated for any of the samples collected for blood volumes of 0.00025 µL.

Therefore, it is observed that when positive results were shown in presumptive testing, DNA analysis also showed results. This indicates, as expected, that presumptive testing is a reliable tool to help determine whether DNA testing will be successful. Additionally, these results demonstrate that presumptive testing can still be performed even after a wet-vacuum collection technique has been utilized.

Comparison to Previous Research

Although a direct comparison with the study performed by Sorenson Forensics [30] is difficult due to the differences within the methods, a general trend between the results they presented and the results presented here is observed. In the research presented here, the wet-vacuum system recovered higher percentages of DNA than the double swab method on the porous substrates (denim and carpet), with the exception of 7.5 µL blood on carpet. While a direct comparison between the double swab method and the M-Vac® was not presented on these same substrates with blood, the Sorenson study reported that the wet-vacuum system was able to recover more DNA than swabbing when collecting blood on nylon, another porous substrate. In addition, it was also observed in the Sorenson study that the wet-vacuum system

recovered higher levels of DNA than swabbing when using saliva samples on other porous substrates, which included denim [30]. This trend in the saliva sample results is consistent with the trend observed with blood within this research and suggests that wet-vacuum collection may be used on a variety of sample types.

While there has not been significant research published in the comparison of a wet-vacuum technique to other collection methods, as is the aim of this research, there has been some comparison between swabbing and taping. In a study performed by Kenna *et al.,* a comparison between the double swab technique and taping, through the use of mini-tapes, was performed for saliva samples on skin. It was observed that taping recovered slightly higher levels of DNA than the use of the double swab method. However, when observing the ranges of DNA recovery, 3.32 – 18.28 ng/µL and 3.60 – 13.71 ng/µL DNA recovered from swabbing and 5.12 – 23.94 ng/µL and 4.74 – 21.27 ng/µL DNA recovered from taping, the overall differences are minimal [46]. In another study performed by de Bruin *et al.,* the double swab method and a taping method (referred to as stubbing) were compared. It was observed that both methods were comparable in practice when collecting epithelial cells on skin and only showed minor differences in the DNA profiles [47]. Additionally, in research performed by Crossler and Bever, different types of swabs and tape were analyzed, including BVDA Instant Lifters®. The results obtained for this study for DNA profiles produced from blood samples on glass, cotton, and paper were

similar overall, with some exceptions. In addition, the BVDA Instant Lifters® produced comparable results to the cotton swabs on all substrates spotted with blood [48]. Specifically, Crossler and Bever examined the number of detected alleles and determined that these numbers did not significantly change between methods.

Although it is important to note that previous methods and specific types of materials tested may not have been the same as in the research presented here, the results shown in this research also demonstrate that the double swab method and taping were not significantly different. Where one method may have performed better in one area of analysis, it was outperformed in another; again, this suggests a new collection technique for low-template samples may benefit the forensic community. Introduction of a new collection technique for certain suspected low-template samples would allow more DNA to be collected, thereby increasing DNA profiling capability from such samples, which traditionally would have been considered inappropriate for DNA purposes. However, given the ability and power of the wet-vacuum technique, consideration as to potential background interference needs to be assessed when choosing between methods.

Conclusions

While method-substrate comparisons can be made through the calculations of DNA percent recovery, MDS, and LOD, it is important to assess all of these variables simultaneously to make a sound conclusion as to which collection technique is optimal. While most of the conclusions are based on the data analysis presented in the research here, the presumptive testing study was also taken into consideration. It is also necessary to consider the ease of use with respect to each collection method and the presence of potential background DNA on the substrate.

When deciding which method to use, the primary aspect to consider is not whether the substrate is non-porous or porous, but whether the biological evidence is visible or not. If the stain is located on a non-porous surface and is clearly visible (i.e. there is likely a significant level of DNA) then the optimal collection technique would be the double swab or taping method. These techniques would provide an analyst with a fast, easy collection and sufficient DNA recovery. This reasoning also holds true for a visible stain on a porous substrate. Like on the non-porous surface, this situation suggests the analyst can efficiently use the easier and faster collection methods available. In this case, either of these two methods (double swab or taping) would provide an analyst with sufficient collection of the biological stain. While the taping method had lower LODs on porous substrates, the double swab method made up for this with higher MDSs.

If the stain is not visible, it is then suggested that the analyst consider whether or not the likely location of the stain is known. If a biological stain's location is known and within a confined area, and it is suspected that ample DNA is present, then the same recommendations apply as if the stain was visible on the substrate. Thus, the optimal collection method to utilize in this instance would be the double swab or taping method. Due to the known location and high concentration of the sample, utilizing the wet-vacuum system would be unnecessary when a more rapid and less labor-intensive technique would be sufficient.

However, if only the general location of the stain is known and it was deposited over a larger surface area, then the use of the wet-vacuum may provide the analyst with a more efficient way to collect than taping or the double swab method. This would be especially true when the evidence is suspected to be present in lower concentrations. It would be less difficult to collect from this larger area using the wet-vacuum system than using an abundance of swabs or tape pieces to ensure enough evidence was collected for downstream processing. Although the LODs calculated from the M-Vac® collections on denim and carpet were not as low as the double swab or taping methods, the ability to cover large areas and the percent DNA recovery shown for smaller blood volumes in this study support the wet-vacuum collection's value in this type of situation.

If the location of a non-visible stain is unknown or if it is thought to be very large and spread out on the substrate, it is suggested that collection be performed with the wet-vacuum system. Although other methods were shown to sufficiently collect DNA, and generally maintained equal or better results with respect to their MDSs and LODs on the different substrates tested, the very nature of this situation implies that the taping and double swab methods may not provide a sufficient collection in order to avoid negative results in downstream processing. Without the knowledge of the approximate location of the stain, the wet-vacuum system automatically lends itself to being able to cover a large surface area, while the double swab and taping methods do not. Ultimately, the use of the wet-vacuum would increase the probability that sufficient biological evidence would be collected. While the LODs calculated for the M-Vac® on denim and carpet were higher and the MDSs calculated were lower than that of the double swab and taping methods, the overall ability of the wet-vacuum system to collect the nuclear material itself would overcome these numbers.

However, when collecting using the wet-vacuum, traffic and/or the presence of background DNA must also be taken into consideration. High-traffic areas may contain DNA from an innumerable number of contributors. If the stain is visible, then, as previously described, swabbing or taping is recommended. However, if the stain is not visible or spread over a large, high-traffic area, DNA results, although present, may be considered too complex for interpretation.

Therefore, appropriate DNA interpretation guidelines for complex, low-level mixtures may be required for results originating from these areas.

Figure 16 demonstrates a visual representation of the final recommendations for when each biological collection method – the double swab method, taping, or the M-Vac® – would be most optimal.

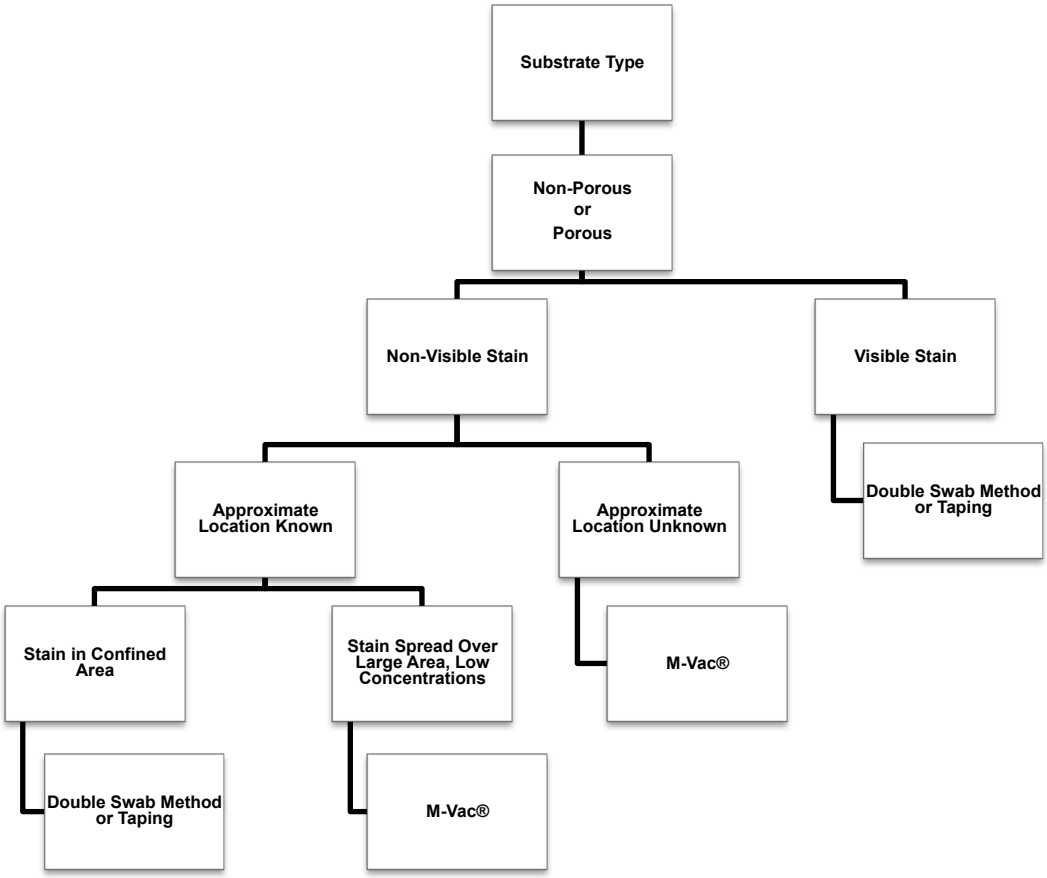

Figure 16. *Suggested recommendations for use of biological evidence collection methods: the double swab method, taping, or the M-Vac®.*

Future Work

While current research in pathogen testing and forensics has explored various aspects of wet-vacuum collection, examination into the comparison of the collection techniques discussed here on additional substrates and with various other body fluids is required. Additionally, utilizing different biological collection methods with different sizes of substrates would give insight into the total surface area that the wet-vacuum is able to cover as compared to other methods. It may also be of value to conduct more research on the effect of possible contamination or background noise observed in STR profiles following collection with a wet-vacuum system. These types of studies would allow for further optimization of the M-Vac® wet-vacuum system as a forensic biological evidence collection tool.

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List of Journal Abbreviations

Anal. Bioanal. Chem.	Analytical & Bioanalytical Chemistry
Anal. Chem.	Analytical Chemistry
Biotechnol.	Bio/Technology
Environ. Sci. Technol.	Environmental Science & Technology
Forensic Sci. Comm.	Forensic Science Communications
Forensic Sci. Int.	Forensic Science International
Forensic Sci. Int.-Gen.	Forensic Science International: Genetics
J. Forensic Sci.	Journal of Forensic Sciences
Methods Enzymol.	Methods in Enzymology

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Curriculum Vitae

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Master of Science in Biomedical Forensic Sciences
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Saint Mary's College, Notre Dame, Indiana
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Research

Improving DNA Evidence Collection via Quantitative Analysis: A Systems Approach, Master's Thesis, Boston University (BUSM), Boston, Massachusetts, Program in Biomedical Forensic Sciences

Annotation of contig49 and contig28 of the *Drosophila mojavensis* **Dot Chromosome,** Undergraduate Research for Senior Comprehensive, Saint Mary's College, Notre Dame, Indiana

Professional Experience

Graduate Research Assistant, Boston University (BUSM), Boston, Massachusetts, Program in Biomedical Forensic Sciences, Spring 2013

Undergraduate Teacher Assistant, Saint Mary's College, Notre Dame, Indiana, Department of Biology, Spring 2010

Awards and Achievements

Kappa Gamma Pi Honor Society, The National Catholic College Graduate Honor Society, Baccalaureate Membership, Spring 2011-Present

Beta Beta Beta – National Biological Honors Society, 2009-Present

Presidential Scholarship, Academic Scholarship, Saint Mary's College, Notre Dame, Indiana, 2007-2011

International Baccalaureate Diploma, Portage Central High School, Portage, Michigan, 2007

Presentations

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