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Evaluating the Use of the M-Vac® Wet Vacuum System to Recover DNA from Cotton Fabric

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Evaluating the Use of the M-Vac® Wet Vacuum System to Recover DNA from Cotton Fabric

Phillip Reilly Irion

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Phillip R. Irion, B.S.

**Thesis submitted to the Eberly College at West Virginia University
in partial fulfillment of the requirements for the degree of
Masters of Science in Forensic and Investigative Science**

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**Morgantown, West Virginia
2020**

**Keywords: M-Vac®, Sample Collection, Wearer DNA Forensic DNA Analysis
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ABSTRACT

Evaluating the Use of the M-Vac® Wet Vacuum System to Recover DNA from Cotton Fabric

Phillip R. Irion, B.S.

With the current state of the field of forensic biology, collecting DNA from evidence samples may pose some difficulty. Traditional methods for collection of DNA from biological fluids and touch samples include cuttings, scrapings, and the double swab method. While all of these methods have benefits, they also have their drawbacks. In order to more efficiently collect DNA from an evidence sample, novel approaches need to be explored. This novel approach is the microbial vacuum (M-Vac®) Wet Vacuum System.

This system utilizes a sterile collection solution and a vacuum to collect DNA material from evidence samples. While it is similar to the double swab method, it takes collection a step further by collecting DNA material within the pores of samples. It is especially beneficial for cold cases where DNA material from the surface of a piece of evidence was previously collected but there was still trace DNA trapped in the pores. This method eliminates issues related to sample size and attempting to determine where to collect on a piece of evidence. It also has the benefit of sampling evidence areas directly and in its entirety. This can decrease the number of evidence samples needed to be transported to the laboratory for processing since the M-Vac® can be used at crime scenes. Therefore, this system is beneficial for its use as a serological tool for a crime scene analyst and a DNA analyst.

Some preliminary research has been performed by the Moroose Research Lab comparing the M-Vac® to traditional cuttings for collection of blood, saliva, and semen, and wet swabbing for touch DNA on unworn and 15-minute worn cotton t-shirts. The unworn study showed that the M-Vac® might be beneficial with the collection of touch DNA as compared to swabbing. As for the 15-minute worn study, it was shown that background wearer DNA has the potential to interfere with the interpretation of an evidence sample's electropherogram when collected with the M-Vac® by being preferentially amplified over DNA from the evidence sample. However, with t-shirts being worn for 15 minutes is not very indicative of a true crime scene scenario. So, to determine the extent of interference wearer DNA can have on an evidence sample's electropherogram when collected with the M-Vac®, a study with t-shirts worn for 12 hours was conducted.

This study was the same as the previous worn study conducted with the only difference being that the t-shirts were worn for 12 hours instead of only 15 minutes. T-shirts were worn by individuals for 12 hours consecutively and then a biological fluid or touch DNA was placed onto the worn t-shirt. Fluids were either blood, saliva, or semen. This new study helped to fill the gap of knowledge from the previous 15-minute worn study by determining the extent wearer DNA had on the quantity of human DNA and the electropherograms for evidence samples collected with the M-Vac® and traditional collection methods, such as a cutting or a wet swab, after a participant wore a t-shirt for 12 hours consecutively. Initially, based on the DNA quantity data, the M-Vac® was deemed to be less efficient than the traditional method of cuttings for the collection of blood, saliva, and semen, but more efficient for the collection of touch DNA. However, when reviewing the electropherograms, the M-Vac® outperformed the traditional method of cuttings for the collection of blood, saliva, and semen, and the traditional method of wet swabbing for the collection of touch DNA. Yet, this does come with one drawback. As the amount of wearer DNA starts to increase in quantity to either match the quantity of an evidence sample or exceed the quantity of an evidence sample, a mixture profile occurs and it becomes harder to reliably determine who the contributor is for the sample and the person who wore the t-shirt, especially if both sources are unknown.

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1. Introduction

Within the past few years, the field of Forensic DNA Testing has improved in both methodology and instrumentation in the laboratory for the detection and sensitivity of Short Tandem Repeats (STRs). This has helped immensely with increasing turnaround times, sensitivity, and processing. Yet, there is still a major issue for the crime scene investigator: an efficient collection method for DNA. Although initially it seems to be more of a direct issue on a crime scene investigator, how and what a crime scene investigator collects from a crime scene does directly affect evidence processing in the laboratory for a DNA analyst. One specific problem is the collection of touch DNA from evidence samples.

There are a few different methods of collecting DNA from an evidence sample that can be used both at the scene of a crime and in the laboratory. Some of these methods include using a wet swab, taking a cutting, and scraping the evidence, all of which have their advantages and disadvantages. For a wet swab, while it controls the collection of DNA to the swab itself, trying to collect DNA from a large piece of evidence can drastically increase the number of samples to be processed, especially if the double swab method is used. As for a cutting, one can eliminate the secondary transfer and possibly loss of DNA from evidence by sampling evidence itself, but if a sample is not easily visible, such as with touch DNA, it can be hard to determine where to take a cutting from. Scraping has the advantage of sampling a larger area, but the disadvantage comes from potentially a loss of DNA. However, there is another option for collecting DNA from evidence that has many of these advantages and combats all these disadvantages.

The other option for collecting and recovering DNA from evidence is through the M-Vac®. This instrument collects DNA material by spraying an evidence sample with a sterile collection fluid and then uses a vacuum to re-absorb the fluid. Once the collection fluid is vacuumed, it is stored in a sterile collection bottle. This collection fluid is then filtered with a concentration filter. This filter can then be cut out and processed in the same fashion as a swab normally would be in a forensic DNA laboratory.

The M-Vac® system uses the same premise as a wet swab to collect DNA in an aqueous environment, but it goes one step further. The sterile collection solution actually penetrates into the pores of an evidence sample and ultimately vacuumed up by the M-Vac®. This potentially increases the amount of DNA that is collected when compared to the traditional swab technique. This may be beneficial for the collection of trace amounts of DNA trapped in the porous surfaces of evidence. This could be beneficial for cold cases where all the DNA evidence from the surface was previously collected but there could still be trace DNA trapped in the pores. With this method, it eliminates issues related to sample size and attempting to determine where to collect samples on physical evidence. It also has the benefit of sampling evidence areas directly and in its entirety. This can decrease the number of evidence samples needed to be transported to the laboratory for processing since the M-Vac® can be used at crime scenes.

This instrument has been utilized in previous casework, has been validated both externally from the M-Vac® company itself and internally from the company, and previous research has been conducted by universities. In previous casework, this instrument has been used when collection of DNA from evidence via traditional methods, such as the double swab method, did not yield a DNA profile. Some examples of evidence processed by the M-Vac® that produced a full or partial profile includes: clothing, either years old or soaked in blood; items soaked in water; cold case evidence items; and blunt objects, such as bricks and stones. As for the previous validations of this instrument, it has been externally validated by the Richland County Sheriff Department, the Washington State Patrol, and the Philadelphia Police

Department. These external validation methods included testing various substrates, sample types, and using the M-Vac[®] after evidence samples had been recovered via traditional collection method. As for internal validation, this instrument was validated with samples to include blood, touch DNA, and urine on various substrates, and the validation of the concentrate from the filter. As for the previous research, four different universities and one police department have conducted various research projects on this instrument. Cedar Crest College conducted research on collection of DNA from finger prints after staining and on touch DNA from different fabric materials. West Jordan Utah Police Department is currently working on their Bardole DNA collection method using the sterile collection fluid and the concentration filter to collect DNA from small objects like bullet casings. Another university that has conducted research on the M-Vac[®] was UC Davis. Two of their studies consisted of using the M-Vac[®] to collect saliva from the skin pre and post a participant showering, and their third study comparing the M-Vac[®] to the traditional cutting methods for collection of touch DNA on a t-shirt worn for 8 hours. Boston University was the third university to conduct research with the M-Vac[®]. Their research first focused on the comparison of the M-Vac[®] versus double swabbing or taping traditional collection method for blood on denim fabric, and then second on the collection of blood and semen on different substrates with just the M-Vac[®]. The last university to conduct research on the M-Vac[®] was West Virginia University (WVU). A preliminary study was conducted on both unworn and worn t-shirts with blood, saliva, semen, and touch DNA. In this study, the M-Vac[®] was compared to either cuttings or swabs and the t-shirts were worn for 15 minutes.

The goal of this proposed research is to gain knowledge on whether the M-Vac[®] system is more effective than traditional methods, such as cuttings and swabbing. This would specifically be for the collection of DNA from blood, saliva, semen, and touch DNA from a piece of evidence that may be found at a crime scene, a cotton t-shirt. This is novel from previous research from other universities and it is an expansion of the previous research performed at West Virginia University since the t-shirts will be worn for 12 hours instead of 15 minutes, which will yield a better understand of this research on case-work type samples and how wearer DNA can effect interpretation of electropherograms This proposed research would help to determine if the M-Vac[®] is of viable use on biological fluids deposited on cotton fabric. It could aid an analyst in the best collection method for obtaining a DNA profile when a biological sample has been deposited on a t-shirt worn for 12 hours.

2. Background

2.1 Deoxyribonucleic Acid

Deoxyribonucleic acid (DNA) is commonly known as the genetic “blueprint” to life. DNA is typically found in the nucleus of human cells and consists of 22 pairs of autosomes and a pair of sex chromosomes. One of these chromosomes from each of the 22 pairs and a sex chromosome are inherited from both a mother and a father, individually. A person’s mother passes down an X-chromosome and a person’s father passes down an X- or Y-chromosome.

Genes are located on these chromosomes. These genes can code for different proteins depending on the location and function of a cell. Genes on a chromosome are specifically labeled by their location are known as loci. These loci can be in various forms, also known as alleles. When a person’s two alleles are the same, then a person is considered homozygous for a locus; when they are different, a person is considered to be heterozygous for a locus. An example of this terminology related to a homozygous allele can be seen in figure 1.

Figure 1. Example of homozygous alleles.¹ Both loci are heterozygous for their alleles. They have a different number of repeat regions for both copies of a locus, which were each inherited from a person's mother and father. When these chromosomes from the two parents are together, they are known as homologous chromosomes.

As stated previously, DNA is essentially the same throughout all the cells a person has, with the exception of sex cells (sperm and eggs) and mutations acquired throughout life. These cells only contain half of the chromosome pairs and one of their respective sex-chromosomes, X is found in eggs and Y or X in sperm. Additionally, a person's DNA is unique to that person, with the exception of identical twins.

The structure of DNA is composed of four nucleotides bases—adenine (A), Thymine (T), cytosine (C), and guanine (G), and a backbone composed of a deoxyribose sugar molecules and phosphate groups. Adenine and guanine are known as purines and are composed of two cyclic groups. Thymine and cytosine are known as pyrimidines and are composed of one cyclic group. In its natural state, DNA forms a double stranded helix with two strands that are anti-parallel (i.e. running in opposite directions to one another). To hold together DNA vertically, the phosphates in the DNA backbone form a phosphodiester bond between a 5' carbon of one deoxyribose sugar and the 3' carbon of another deoxyribose sugar. Branching from the deoxyribose sugars, the nucleotide bases hold together DNA's structure horizontally; A binds with T via two hydrogen bonds, while C binds to G via three hydrogen bonds. Figure 2 is a representation of a DNA molecule.

Figure 2. Structure of DNA.² DNA is a double helix molecule that runs in an anti-parallel fashion. The backbone of DNA is composed of sugars and phosphates. Nucleotide pairings, A to T and C to G, are what holds two strands together. Base pairing between A and T nucleotides are formed with two hydrogen bonds, whereas base pairing between C and G are two hydrogen bonds.

2.2 Polymorphisms

Approximately 5% of the human genome is used for protein coding.³ The rest is composed of non-coding regions that have essentially no effect on the coding regions. Therefore, this can allow mutations and variations to build up over evolutionary time without having a deleterious effect. These mutations and variations give rise to highly variable regions, known as polymorphisms.³ These polymorphisms can be used by a forensic biologist to differentiate one person from another, with the exception of identical twins.

There are three main types of polymorphisms: single nucleotide polymorphisms (SNPs), minisatellites, and microsatellites. Additionally, these polymorphisms can be either sequence or length based. The former is based on the DNA sequence itself and the latter is based on a number of repeat regions in a sequence, usually 3-4 base pairs. SNPs are self-explanatory for they are variations in a DNA sequence at one base pair and are sequence polymorphisms. Minisatellites and microsatellites are both length polymorphisms. These two only differ based on the range of base pair that a length polymorphism has; minisatellites tend to have 8-100 base pairs and microsatellites 2-7 base pairs.¹ Microsatellites are commonly known as short tandem repeats (STRs) and are used by forensic biologist's to generate DNA profiles for differentiation of one person from another.

2.2.1 STR Profiling

The current gold standard for forensic biology for human identification are STRs. Within the past few years, forensic biologists in the United States only examined 13 core loci per the

FBI standards for participating in the Combined DNA Index System (CODIS).⁴ However, as technology and reagent kits developed, the number of loci examined was increased to 20. The loci examined are composed of four base pair sequences that have a set range of repeats that can occur. The overall process to obtain an STR profile includes extraction, quantitation, amplification, separation, and detection.

2.3 Extraction

The process of DNA extraction isolates nuclear DNA from a cell and from a cell's other components (i.e. RNA, proteins, lipids, and other organic and inorganic materials). This is done to ensure that only DNA is being carried through the processes to generate an STR profile and that anything that would inhibit these processes is removed. Additionally, this ensures the removal of nuclease proteins and other things that could lead to the degradation of DNA while it is being stored. There are a multitude of ways to extract DNA, which are based on the source, age, size, and degradation of a sample. For DNA laboratories, common sources of DNA include saliva, blood, semen, vaginal swabs, hair and bones.

In order to isolate nuclear DNA from a cell, a cell must first be lysed with a solution that contains a salt, detergent and proteases. These break down the cell and nuclear membranes, and denature any proteins within the cell, respectfully. This exposes DNA, which can then be purified from everything else in a cell lysis mixture. Kits commonly used for nuclear DNA extractions include: sodium chloride salts, tromethamine (TRIS) buffer, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), and proteinase K.¹ A commonly used kit within forensic DNA laboratories is the QIAamp DNA Investigator Kit, which uses a series of spin steps and a DNA binding column to separate free nuclear DNA from other cellular components. This isolated nuclear DNA can then be quantified.

2.4 Quantitation

Once nuclear DNA has been extracted from a sample, the amount of nuclear, human DNA has to be quantified per FBI Quality Assurance Standards.⁴ Additionally, this step is necessary to determine the concentration of human DNA because a sample must be either diluted or concentrated to 0.5-2 ng/uL of DNA for commercial DNA amplification kits.¹ If a sample has too much DNA, then when a sample is amplified, polymerases will not have enough time to properly incorporate 3' A nucleotides at the ends of all the amplicons. This will result in split peaks on an electropherogram.¹ Additionally, it can result in other artifacts on an electropherogram such as off-scale alleles, pull-up and stutter.¹ Having too little DNA can lead to little to no data, preferential amplification of loci, locus imbalance, and allelic dropout.¹

The process used to commonly quantitate the amount of DNA present in a sample is real-time polymerase chain reaction (PCR), also known as quantitative PCR (qPCR). This process monitors the concentration of single stranded, template DNA in real time by the amount of fluorescence yielded. There are 4 phases to this process: baseline, exponential, linear, and plateau.¹ For the baseline phase, there is not enough fluorescence being detected by the instrument to distinguish it from background noise.¹ Then in the exponential phase, fluorescence of DNA can start to be distinguished from background noise and there is an optimum amount of reaction components.¹ During this phase, the cycle threshold (C_T) is reached, which is the number of cycles needed for fluorescence to cross a set threshold, which is determined by a standard curve.¹ This threshold is the point where the amount of fluorescence is inversely proportional the concentration of DNA and therefore the fluorescence of DNA can be

distinguished from background noise.¹ A lower C_T value indicates a higher concentration of DNA because it took less time to reach a set threshold, whereas a low C_T value indicates a low concentration of DNA.¹ For the linear phase, the efficacy of the process starts to decline because one or more of the components of PCR have decreased to below their optimum levels.¹ So, the slope of fluorescence starts to decrease. Finally, with the plateau phase, all components of the PCR process have been depleted below optimum levels and no more fluorescence is detected by an instrument.¹ All of this can be seen in figure 3.

Figure 3. qPCR fluorescence plot.¹ The process of qPCR has four phases: baseline, exponential, linear, and plateau. The C_T values on this figure is the amount of fluorescence needed to pass a set threshold. This threshold is the point at which the amount of fluorescence is inversely proportional to the concentration of DNA present.

During this process, unknown samples are run with a set of serial dilutions of a known DNA standard. These standards also yield C_T values based on their respective concentrations. From this, a standard curve can be made. This allows for the approximate concentration of DNA from an unknown sample to be determined from its C_T value and the standard curve that was generated.

The commonly used kit in forensic DNA laboratories is the Quantifiler™ Trio Kit, which targets a small autosomal region, a large autosomal region, and a male specific (Y Chromosome) region. In a normal, non-degraded, DNA sample, the large autosomal region is used for either concentrating or diluting a DNA sample. Typically, these kits are based on a 5' nuclease assay process and contains taqman probes, dinucleotides, Amplitaq Gold DNA polymerases, and forward and reverse primers specific to target regions.¹ The taqman probes have a 5' fluorogenic dye, a 3' quencher molecule, and a 3' minor groove binding (MGB) domain.¹ These probes binds to DNA sequences that are flanked by primers. When the fluorogenic dye and the quencher molecule are close together on the probe, the fluorescence is quenched.¹ However, when the taqman probe is broken down by the Amplitaq Gold DNA polymerase, they separate and fluorescence can be detected.¹ The MGB domain assists with keeping the taqman probe in place until it is broken down by the polymerase and increases the melting temperature of the probe without increases the length of the probe.¹ The polymerase in these kits have a 5' endonuclease activity, which is what is used to break down the taqman probes and leads to the detection of fluorescence.¹ This whole process with the taqman probe can be seen in figure 4. As the single stranded DNA templates form double strands through the qPCR process, the amount of

fluorescence increases till there are no longer any single stranded templates left. At this point, the DNA can be amplified once it been concentrated or diluted appropriately if need be.

Figure 4. Taqman probe reaction.¹ The taqman probe binds to a single stranded, sequence of DNA between to primers. The forward primer of the set acts as a docking station for Amplitaq Gold DNA Polymerase to bind to DNA and then elongates to form double stranded DNA. During these elongation step, the 5' endonuclease activity breaks down the bound Taqman probe and releases the fluorogenic dye. When the fluorogenic dye is not in close contact with the quencher dye, it fluoresces.

2.5 Amplification

PCR can be also used to exponentially copy a sequence from a sample of DNA.¹ Typically, this process occurs with a series of steps that involve heating and cooling. More specifically, this process denatures the DNA, anneals primers, and extends a DNA sequence with an enzyme polymerase and dinucleotides.¹ During the denature step, the typical temperature is 94°C; at this temperature DNA unwinds to single strands, which leaves DNA sequences accessible to molecular machinery.¹ Then for annealing, forward and reverse primers bind just outside of sequences of interest at 50-60°C.¹ Finally, the temperature either stays constant or raises to 72°C to allow for DNA polymerase to bind and extended a DNA sequence with dinucleotides.¹ This whole process is usually repeat 29-40 times depending on the end application of the PCR products.¹

One typical kit used for forensic DNA laboratories for this process is Globalfiler™ PCR Amplification Kit. This kit contains the components already listed. Its primers target for the 20 loci used in CODIS, amelogenin for sex determination, two male specific loci and the SE33 locus. Once DNA samples are amplified with this kit, the amplicons can be separated and the repeat units or alleles for the loci can be detected.

2.6 Separation and Detection

The process of capillary electrophoresis is used to separate STR amplicons on a genetic analyzer instrument, one such instrument is the 3500 genetic analyzer.¹ Negatively charged DNA fragments are separated by size through a narrow capillary tube that has a sieving polymer in it.¹

Fragments that are small will run faster through the capillary and those that are larger will run slower because of this sieving polymer.¹ An electrokinetic injection is used to move DNA from a negatively charged reservoir into a capillary.¹ These DNA fragments then move to the other end of the positively charge capillary and are collected.¹

Fluorescent tags are placed on the STR amplicons during the PCR amplification step.⁴ These tags are excited by a 505nm laser in the detection window of a capillary and a series of lenses focus the light to a spectrograph, which contains a diffraction grating to disperse light based on the wavelengths.¹ These wavelengths are then focused onto a CCD array to filter for specific wavelengths of light.¹ This process therefore allows for the separation of STR amplicons based on both size and fluorescence.

The result of this process is an electropherogram, which is used for interpretation of STR profiles, but in order to generate an electropherogram, some processing needs to occur first. Fragments separated by time are converted to base pair length sizes via an internal sizing standard, which is a solution that contains base pair ranging in the known typical sizes for STR amplicons.¹ Then the base pairs are converted to allele calls via an allelic ladder, which is a solution containing all possible allele calls for all STR amplicons that were previously amplified.¹ Additionally, STR amplicons are denatured to single strands with formamide, which is also in the Globalfiler™ kit. This is done because only the forward primer used in amplification has a fluorescent tag for detection.¹

Although these procedures and kits seem effective and logical, both have to be validated both by producers and consumers before they are used on case samples in forensic DNA labs.

2.7 Validation

As stated previously, if a forensic DNA laboratory wants to participate in CODIS, they have to follow the FBI Quality Assurance Standards. This also applies to validation of instrumentation and methodologies used within a lab.⁴ Additionally, the Scientific Working Group on DNA Analysis Methods (SWGDM) also states that new instrumentation and methodologies must be validated first before they are to be used on real case evidence samples.⁵ There are quite a few different attributes that have to be considered for validation, one such one is mixture samples.

2.7.1 Mixtures

DNA mixtures can also have an effect on both instrumentation and methodology, so it is considered for validations within forensic DNA laboratories as well. DNA evidence samples are not always a single source from crime scenes. In fact, a lot of DNA samples have multiple contributors, especially when it come to the collection of background DNA from an evidence item. So, it is important to understand how evidence can be affected when multiple contributors are present.

2.8 Traditional Collection Methods

There are several different ways to collect DNA from a piece of evidence. The collection method used should be chosen in order to reduce the redundancy in evidence collection.¹ Some traditional collection methods include swabbing, cutting, and scraping. Depending on what the evidence is, one collection method can be more efficient than others.

2.8.1 Swabbing

Swabbing of evidence items is a common method used to collect evidence. There are a multitude of different swabs that can be used for collection and the type used depends on the evidence an individual is trying to collect. Two common swabs that can be used to collect DNA are cotton and nylon flocked swabs.⁶ The type of swab used can have implications of the extraction process a DNA analyst should use.⁶

There are a few different ways in which DNA can be collected using a swab. This includes a dry swab by itself, a wet swab by itself, and the combination of a wet swab followed by a dry swab (i.e. double swabbing).⁷ When collecting DNA from an evidence sample, it has been shown that the double swab method is the most efficient at collecting DNA from evidence.⁸

2.8.2 Cutting

Another technique that can be used to collect a biological sample from evidence is through cuttings. Typically, this method is used when a potential biological stain can be seen with the naked eye or with the use of an alternative light source (ALS). For example, if an evidence sample is a t-shirt or something else that can easily be cut with sterile scissors, then a piece from the evidence can be collected and DNA can be potentially obtained from it.⁹

2.8.3 Scraping

Yet another technique that can be used to collect DNA from an evidence sample is by scraping the evidence. This is when a sterile scalpel is used to scrap a piece of evidence.¹⁰ The scrapings are collected on a piece of sterile paper and then the paper is processed like a cutting or a swab would be processed for DNA extraction.¹⁰

2.9 M-Vac® Wet Vacuuming System

The Microbial Vacuum (M-Vac®) Wet Vacuum system is a wet vacuum system that was developed by Dr. Bruce Bradley.¹¹ The M-Vac® was originally designed to be used by the dairy and food industry for food safety in order to potentially collect surface pathogens from meat products.¹¹ He designed this instrument in order to try to collect pathogens from the entire surface of meat products instead of just a small portion of them.¹¹ This is a similar issue for collection of DNA from large evidence samples. So, it was easy to see the potential use of this instrument as a collection method instead of traditional collection methods for DNA, especially with touch DNA.

2.9.1 Part of the System and How It Works

There are several different parts to the M-Vac® instrument itself and the system as a whole. One of the first components of the instrument is the sampling head and the collection bottle. The head sprays a collection solution onto a sample and then vacuums a sample back into a sterile collection bottle, as seen in figure 5.¹²

Figure 5.¹² Image of the sterile collection head attached to the sterile collection bottle.

When the sterile collection solution is applied it helps to loosen DNA and cells in the pores of a substrate. Then when the vacuum is applied, the sampling head collects DNA and cells now suspended in the collection solution. The combination of the sterile collection solution and the vacuum creates a turbulent environment to allow for collection of DNA and cells, as seen in figure 6.¹² This is effective to collect DNA on the surface of evidence and in the pores of a porous piece of evidence. Large and small pieces of evidence can be sampled in its entirety since the collection bottle is interchangeable and can collect up to a measurement of 175mL.¹²

Figure 6.¹² Image of the turbulent environment that the sampling head creates with the combination of the sterile collection fluid and the vacuum.

The next component of the M-Vac[®] instrument is the support equipment case (SEC). This portion of the system contains the control panel for the instrument itself. The control panel controls the hinged pressure chamber to pressurize the collection solution and the vacuum portion of the instrument.¹² In addition, this case also has a holder for the sampling head and the collection bottle so the sampling head can remain sterile if it is going to be used multiple times on a piece of evidence.¹² An image of this can be seen in figure 7.

Figure 7.¹² Image of the SEC.

As previously mentioned, the sterile collection solution is the reagent used by the M-Vac[®]. This solution is both DNA and RNA free, so it allows for the collection of DNA and cellular material without contamination.¹² When this bag is pressurized by the control panel of the SEC, it allows the sampling head to efficiently and consistently spray an evidence sample.²¹ The sterile collection solution can be seen in figure 8.

Figure 8.¹² Image of the sterile collection solution.

The last component of the instrument itself is the extension tubing. This tubing connects the SEC and the sterile collection solution to the sampling head via a dual lumen tube to allow the sampling head to spray and vacuum a sample at the same time without contamination to the sterile collection solution pouch.¹² This tubing can come in multiple lengths to increase the mobility of the instrument for the user.¹² An image of this tubing can be seen in figure 9.

Figure 9.¹² Image of the extension tubing.

Together, these parts allow for the collection of DNA and cells from different pieces of evidence. When the instrument is first turned on, sterile collection solution is brought to the appropriate pressure. While the instrument is adjusting the sterile collection solution to the appropriate pressure, the solution itself is displaced through the extension tube to the sampling head. The sterile collection solution can then be sprayed onto a sample, which is then vacuumed up with any cellular material or DNA. When the collected solution is vacuumed, it is transferred to the sterile collection bottle, as stated previously. A diagram of the instrument and how it works can be seen in figure 10.

Figure 10.¹³ Diagram of the M-Vac[®]. The different parts of the instrument include the sterile collection solution (1), the extension tubing (2), the sampling head (3), the sterile collection bottle (4), and the support equipment case (5).

After an evidence sample has been sprayed and the solution is collected, it needs to be concentrated onto a substrate for DNA extraction. The first step of concentrating DNA and cells from the collection solution is the use of a pre-filter system (PF Shield). This system is typically used to remove excess debris that can be collected during initial sampling of evidence. This filter traps debris larger than 40 μm .¹² Although it is designed to remove debris, this filter can trap some cells, however, it helpful to prevent downstream issue with DNA processing.¹² The PF

shield does not have to be used if the collection solution does not appear to not have very much debris in it.¹² An image of the PF shield can be seen in figure 11.

Figure 11.¹² Image of the pre-filter system.

The concentration filter apparatus is the final step to concentrate DNA and cells for DNA extraction. The apparatus is essentially two collection bottles separated via a filter. This filter is composed of polyethersulfone (PES), and the filter size can be either 0.45 or 0.20 μm .¹² The apparatus is attached to the vacuum and the collection solution is poured onto the filter. When the vacuum is turned on, cells are caught on the filter and the collection liquid is sucked into the bottle at the bottom.¹² It has been previously shown that free DNA might not be able to be concentrated on the filter.¹³ A portion of the filter can then be cut out and processed like a cotton swab would be.¹² The concentration filter can be seen in figure 12.

Figure 12.¹² Image of the concentration filter.

2.9.2 Internal Validations by M-Vac®

M-Vac® System Inc. has performed two separate internal validations. The first one focused on wearer, touch DNA, blood, and urine samples, and the substrates they used included the following: nylon stocking, knit cap, leather purse, cloth glove, shoe, auto trunk lining, duct tape bindings, concrete, skin, cotton cloth bedding, rock and rough landscaping tile.¹⁴ From this first validation, most of the results were similar to previous results from studies done in the past

and from casework samples.¹⁴ Two of their findings stood out from previous findings. First, was that a full profile could be obtained from touch DNA on duct tape, and second, that a full profile was obtained from urine on cotton bedding.¹⁴ Additionally, this validation determined that this process using the M-Vac[®] did not have any downstream consequences for DNA processing.¹⁴ The outcomes from this initial study allowed this instrument to be used as a tool in casework.¹⁴

The other internal validation conducted was a verification of the concentration filter. A 0.45 μm PES filter was used to concentrate saliva evidence on cotton and compare it to the traditional double swab method.¹⁴ In addition, after samples collected with the double swab method, the M-Vac[®] was used to determine if DNA could still be collected afterwards.¹⁴ The filtrate collected from the PES filter was also collected for testing.¹⁴ All of these samples were extracted and the DNA quantities were compared. For the results, the M-Vac[®] collected 39 times more DNA than the double swabbing method in general and 22 times more on samples that were previously swabbed than the previously used double swabs.¹⁴ As for the filtrate, it showed that all the sample collected on the filter as these samples contained 0 ng of DNA.¹⁴ However, one thing to note was that it was not determined whether what was collected on the filter was just cell, just DNA, or a combination of both.

2.9.3 Previous Internal Validations from External Entities

There have been three separate internal validations on the M-Vac[®] instrument by different agencies. One validation is from the Richland County Sheriff's Department. They tested this instrument and compared it to either swabs or scrapings on t-shirts and a brick.¹⁴ Both saliva and touch DNA were tested on t-shirts with one t-shirt left as a negative control, and touch DNA was tested on the brick.¹⁴ The M-Vac[®] yielded higher DNA quantities for the t-shirt with saliva and the touch DNA on the brick.¹⁴ The DNA quantities from the brick was 30 times higher for M-Vac[®] than the traditional method.¹⁴ The DNA quantity was higher for the traditional method for the touch DNA t-shirt, but the resulting DNA profile was better for the M-Vac[®] sample.¹⁴ The t-shirt that was used as a negative control did not yield any DNA quantities, and therefore, there were no alleles called in the DNA profile from either collection technique.¹⁴ As for the DNA profile for the brick, the M-Vac[®] sample yielded a full profile and the traditional method only called two alleles.¹⁴ These results therefore validated the M-Vac[®] for DNA collection from porous, textured, and or rough substrates.

Another validation was performed by Washington State Patrol. They tested this instrument versus the double swab method on a rock, cotton t-shirt, pressure treated wood, knit cap, and duct tape.¹⁴ The first three were tested with blood at different concentrations, and the last two were tested for wearer DNA.¹⁴ All the samples yield more DNA with the M-Vac[®] method as opposed to the double swab method.¹⁴ One important outcome of the results was that debris also collect with the M-Vac[®] from the pressure treated wood sample did not interfere with downstream DNA processing.¹⁴ Another outcome was that smaller objects should be sampled in a clean tray and any liquid in the tray that came off any evidence item should also be collected and concentrated.¹⁴

Lastly, a validation was done by Philadelphia Police Department. This department tested the M-Vac[®] in the following situations: buccal cells on highly porous materials, wearer DNA, touch DNA, M-Vac[®] after traditional methods were used, and on mock touch DNA crime scene scenarios.¹⁴ As a whole, the M-Vac[®] collected more DNA from all situations.¹⁴

2.9.4 Previous Casework Where the M-Vac® Was Used

The M-Vac® instrument has been used on a multitude of different cases and the DNA collected yielded both partial and full profiles that were used to help solve a case. Some of the different evidence items that this instrument has been used on include: clothing (years old, blood soaked, or water soaked t-shirts; panties; pants and pant pockets; socks; scarf; ski mask; halter top; and sweatshirt), bedding (comforters, pillowcases, and bed sheets), blunt objects (bricks, river rocks, and cinder blocks), and miscellaneous items (toy stuffed animal, bat handle, zip ties, wig, towel, Molotov cocktail wick, firearm, gun sock, duffle bag and rope).¹⁴ Some of these items were new evidence items, some either had no DNA collected using traditional methods or minimal DNA collected, or some of these items were cold case items that were being reanalyzed.

2.9.5 Previous Research by Cedar Crest College

There have been many different universities to conduct research using the M-Vac®. One such university is Cedar Crest College. They conducted two different studies. One was the study of touch DNA from fingerprints enhanced with Nile Red (ALS lipid stain) and compared the M-Vac® to double swabbing, taping, scraping, and cutting.¹⁴ Their results indicated that the M-Vac® was the best followed by double swabbing.¹⁴

The second study was focused on the collection of touch DNA from various fabrics using the M-Vac®. They tested the following fabrics: Knitted Cotton, Woven Cotton, Gabardine Polyester, Silk Polyester, Nylon, Rayon, Wool, Linen, 95% Cotton/5% Spandex and Acetate.¹⁴ They found that Wool, 95% Cotton/5% Spandex and knitted cotton yielded the most DNA in that order and were all statically significant when compared to the other seven fabrics.¹⁴ From all the samples, 39% had a concentration of at least 20 pg of DNA for amplification, and of the samples amplified, 75% yielded a profile that could be entered into CODIS.¹⁴

2.9.6 Previous Research by West Jordan Utah Police Department

One entity other than a university that performed some research was the West Jordan Utah Police Department. Francine Bardole's research did not use the entire M-Vac® system, it only used the sterile collection solution and the concentration filter. Bardole soaked small evidence items, such as keys, rings, and bullet casings, in the sterile collection solution, vortexed samples, and then filtered the collection solutions on a concentration filter.^{14,15} She is currently working on this technique as are other laboratories with larger sample sizes to determine the sensitivity of the method, especially with small metal objects.^{14,15}

2.9.7 Previous Research by UC Davis

Another university that has performed research on the M-Vac® is UC Davis. They have conducted four separate studies on the M-Vac®. Two of their studies involve the use of the M-Vac® in comparison to the double swab method to collect male saliva from a female participant's skin, which was done to replicate a sexual assault scenario. One study was done without a participant showering and the other was after a participant showered. For the study without showering, three saliva stains were placed on a participant's upper arm, two on one arm and one on the other in concentrations of 50, 25, and 5 uL.¹⁶ The saliva was allowed to dry and the two spots of the one arm were collected either with the M-Vac® or swab to compare the collection methods.¹⁶ The M-Vac® sample on this arm represented the collection with M-Vac® if it was known where a biological fluid was.¹⁶ The M-Vac® was used for the third spot on the other arm, and the whole arm was sampled with the M-Vac® to simulate an unknown location of a

biological fluid on a person.¹⁶ For the results, the only trial that showed the M-Vac[®] collected more DNA than a swab was for the 50 uL samples for the known location saliva samples.¹⁶ This shows that the M-Vac[®] can potentially be used in cases of sexual assault if it is known where the biological fluid is on a victim.¹⁶

As stated previous, UC Davis then did a follow up study to this with eight known locations of male saliva samples in volumes of 50uL on a female participant's skin after she has showered.¹⁷ One side of the body, four spots, was for swab and the other was for M-Vac[®] to again compare a traditional method to the M-Vac[®].¹⁷ The data showed no statistical difference between the two collection methods.¹⁷

The third study by UC Davis was comparing cuttings to the M-Vac[®] for the collection of touch DNA on a worn t-shirt. A female participant wore a t-shirt for 8 hours.¹⁸ After being worn, a male participant touched the t-shirt twice, once without any previous exercise (i.e. dry) for 2 minutes and 45 second of total contact, and once after brief exercise (i.e. sweaty) for 3 minutes and 45 seconds of contact.¹⁸ It was found that there was significantly more DNA collected for the M-Vac[®] samples rather than the cuttings regardless of the touch sample.¹⁸

The last study by UC Davis was a comparison between the double swab method of the M-Vac[®] to collect touch DNA from a strangulation. A strangulation was simulated by having a male participant grab a female participant's upper thigh. A female participant was "strangled" by a male participant for 0, 30, 60, and 90 seconds and touch DNA was collected either directly after strangulation, 3 hours, 6 hours, or 9 hours after strangulation.¹⁹ An extra comparison was done between couples that did and did not live together.¹⁹ The DNA quantities yielded from the collection via the M-Vac[®] were on average not enough to concentrate to 1ng, whereas all the samples collected via the double swab method could be either concentrated or diluted to 1ng.¹⁹ For the samples collected via the M-Vac[®], no male STR DNA profiles could be generated.¹⁹ As for the double swab samples, one produced a full male STR DNA profile, six produced a partial male STR DNA profile, and one did not produce a male STR DNA profile.¹⁹ No significant data was found based on whether or not a couple lived together.¹⁹

2.9.8 Previous Research by Boston University

Boston University school of Medicine conducted three different studies using the M-Vac[®]. One of these studies compared the traditional methods of double swabbing and taping to the M-Vac[®]. They compared these collection methods with differing volumes of blood, 0.075 to 75 uL, on denim.²⁰ The results showed that the M-Vac[®] collected more DNA then swabs or Taping at all concentrations of Blood.²⁰

Another study was conducted with the collection of biological fluids on various substrates with just the M-Vac[®]. The biological fluids tested were blood and semen in various volumes (100 to 0.1 uL), and the substrates were denim, carpet, tile, and brick.²¹ From the largest volumes to the smallest volumes for blood and semen on tile, denim, and carpet, the M-Vac[®] collected enough DNA to concentrate or dilute to 1ng for amplification and capillary electrophoresis.²¹ As for the brick, the M-Vac[®] collected enough DNA to concentrate or dilute to 1ng for amplification and capillary electrophoresis for sample volume at the highest volume tested to as low as 1uL of blood and semen samples.²¹ The sample of blood and semen at a volume of 0.1 uL did not yield enough DNA from the bricks.²¹

Lastly, a study was conducted to determine the spread of the M-Vac[®] collection solution. It was found that DNA could spread from up to 4 inches from the collection site on a piece of

evidence.²² These results therefore suggests caution when collecting samples via M-Vac[®] if two potential samples are close to one another.²²

2.9.9 Previous Research by Purdue University

One study was conducted at Purdue University comparing the M-Vac[®] to double swabbing. The substrate they used was unworn cotton t-shirts and they tested blood, saliva, semen, and urine.²³ It was found that the M-Vac[®] collected about six times the quantity of DNA from blood as compared to double swabbing, and about two times the quantity of DNA from semen.²³ As for the saliva and urine samples, the results were similar between the M-Vac[®] and double swab method.²³

2.9.10 Previous Research by Swedish National Forensic Centre

The Swedish National Forensic Centre conducted a study comparing the M-Vac[®] and swabbing for saliva samples. They collected samples from both nonporous and porous substrates, which included laminate wood, glass, cotton t-shirts, and towels.²⁴ For the wood samples, the M-Vac[®] yielded higher quantities of DNA than were collected via swabbing, and for the glass samples, the two methods were comparable for the quantity of DNA collected.²⁴ When reviewing the results of t-shirts and the towel, the comparison was between the substrates as oppose to the method of collection since only the M-Vac[®] was used to collect saliva from these samples.²⁴ The t-shirts yielded more DNA with the M-Vac[®] than the towel did.²⁴

2.9.11 Previous Research by King's College London

King's College London performed a few studies using the M-Vac[®]. The first study they conducted was determining whether the PES filter was collecting cells or free DNA.¹³ Their results showed that free DNA was in the filtrate and that it was likely that just cells are what are being collected on the concentration filter.¹³

A second study was comparing the M-Vac[®] to the double swab method for collecting saliva from tiles and bricks.¹³ It was determined that the M-Vac[®] recovered approximately 75% DNA from bricks than double swabbing.¹³ As for the tiles, the double swab method collected significantly more DNA than the M-Vac[®].¹³

The last study was comparing the collection of touch DNA from brick using either the M-Vac[®] or double swab method.¹³ When reviewing the results from the obtained DNA profiles, there was not really a difference in the peak heights and number of alleles called between the two collection methods.¹³

2.9.12 Previous Research by the Federal Bureau of Investigation

The Federal Bureau of Investigation (FBI) also conducted a study with the M-Vac[®] where they compared it to a wet swab method for the collection of diluted blood from twenty-two different substrates. These different substrates varied from rough to smooth with different levels of porosity. They found that eighteen of the porous substrates yielded on average 12 times more nuclear DNA with the M-Vac[®] as opposed to using a wet swab.²⁵ For the other four substrates, two porous and two nonporous, the M-Vac[®] and the wet swab method yielded comparable amounts of total DNA.²⁵ The two porous substrates where there was no difference between the methods were cinder blocks and unpainted dry wall.²⁵ As for the two nonporous

substrates, they were glass and a wood countertop.²⁵ In none of the scenarios they tested did the wet swab method yield more DNA than the M-Vac®.²⁵

Within this same study they also wanted to determine if the M-Vac® could collect additional DNA after a substrate had been swabbed with a wet swab. It was shown that it was possible to collect additional DNA using the M-Vac® after a substrate was swabbed with a wet swab.²⁵ Also, they performed an analysis to determine how much mitochondrial DNA was collected with the two methods. Just like the results of the nuclear DNA, the two methods were comparable to one another.²⁵

Overall, their conclusion was that even though the M-Vac® was better for some porous substrates, swabbing is preferred when you can see a biological fluid or if the substrate is nonporous since swabbing is typically more simple, convenient, and cost effective.²⁵

3. Preliminary Studies

A preliminary study was conducted to determine the most successful method for collecting a biological sample from evidence and thus generating a DNA profile. Specifically, the M-Vac® instrument was compared to traditional methods and the evidence sample used was a cotton t-shirt, first unworn and then worn for 15 minutes. The two traditional methods compared to M-Vac® were cuttings and swabs. Cuttings were used for biological samples that contained either blood, saliva, or semen, which is typical when a biological sample can be seen on a piece of evidence. However, since there are three common methods to collect touch DNA from an evidence sample, swabbing, scraping, and cuttings, it needed to be determined which was more effective. The results in appendix A showed that none of the t-test comparisons between any of the collection methods were significantly different in quantity of DNA collected, but the swab method showed the least variation in standard error. So, this was the method used for comparisons with the M-Vac® for touch DNA samples.

When comparing the DNA quantity collected for the M-Vac® versus cuttings for collection of blood, saliva, and semen on unworn cotton t-shirts, the cuttings yielded a significantly higher quantity except for the concentration of DNA for the large autosomal and Y chromosome target for the blood samples as can be seen in appendix B. As for the DNA quantity collected for the M-Vac® versus swabs for the collection of touch DNA on unworn t-shirts, there were no statistically significant differences in concentration for any of the three targets, which can be seen in appendix B. This shows that the cuttings collect more DNA than M-Vac® for biological samples that can be seen with the naked eye or with an alternative light source (ALS). Whereas, the results show that the M-Vac® is comparable to the swabbing collection method in the quantity of touch DNA collected.

The same study was conducted, but with cotton t-shirts worn for 15 minutes. For the samples tested, there were no significant differences observed when comparing M-Vac® versus traditional methods, just as seen with the previous data with the unworn t-shirts for touch DNA. However, this could have likely been due to the background DNA from the wearer increasing the amount of total DNA collected from a t-shirt. So, a comparison was done between M-Vac® or the traditional method and the substrate control from the worn t-shirt. The following data was obtained: for blood, the only significant differences were between cuttings and substrate control samples for the large autosomal and small autosomal targets; for saliva, the only significant differences were between M-Vac® and substrate control samples for the small autosomal target and Y chromosome target, and between the cutting and substrate control sample for the Y

chromosome target; for semen, all t-test comparisons between M-Vac® or cutting compared to the substrate control were significantly different; and for touch DNA, the only t-test comparison that was significantly different was M-Vac® and substrate control samples for the small autosomal target. All of these results can be seen in appendix C. From these results, no trends stand out to explain the data, so a comparison was done between the unworn and the worn t-shirts for the quantity of DNA collected for the different collection methods.

When comparing the unworn and worn t-shirts for blood samples for different collection methods and the substrate controls by themselves, the only t-test comparisons for M-Vac® and cuttings that yielding a significant difference was for Y chromosome target, and the substrate control samples showed no difference between the unworn and worn samples for any target for DNA quantitation. For the saliva samples, the only significant difference was between the unworn and worn t-shirts was for the cuttings for the Y chromosome target. As for the semen samples, the comparisons between the unworn and the worn t-shirts were not significant for the M-Vac® or the cutting methods for any of the targets, but the substrate controls showed a significant difference for all targets. Then for the touch samples, the only significant difference was the small autosomal target for the substrate control samples. All of these results can be seen in appendix D. The comparisons that showed a significant difference between unworn and worn can potentially help to explain why all the M-Vac® versus a traditional collection methods for the worn t-shirts were now no longer significantly different, however since the entire concentration filter was not tested, this cannot be stated with certainty. Additionally, the t-shirts were only worn for 15 minutes, so a longer wear time might yield a difference in the unworn and worn DNA quantities to explain why there is no longer a difference between the M-Vac® and traditional collection methods for DNA quantities.

When reviewing the electropherograms for the samples from unworn t-shirts run for profile analysis, the cutting samples for all of the biological fluids yielded a useable DNA profile. As for the M-Vac® collected biological samples on the unworn t-shirts, six of nine electropherograms had full useable DNA profiles. With the substrate control samples for the unworn t-shirts, four of the five were appropriately blank with one substrate control showing low level female contamination. As for the touch samples from the unworn t-shirts, the following results were obtained: only one swab sample of the four processed yielded a full DNA profile; three of the four M-Vac® samples yielded a full profile, but two of those had low levels of contamination; and the substrate control showed low levels of male contamination. These results and an example of the D3S1358 locus for the unworn and worn data for the different biological fluids and touch DNA for both the M-Vac® and traditional methods can be seen in appendix E.

When reviewing the electropherograms for the samples from worn t-shirts processed for DNA profiles, all of the cutting samples for the blood samples yielded a useable DNA profile with alleles called for the wearer of the t-shirt ranging from none to 10 alleles calls. As for the cuttings for the semen samples, half of the samples yielded a usable DNA profile with none to 4 alleles called from the wearer of the t-shirt. For the cuttings for the saliva samples, all samples yielded a usable DNA profile with only one sample having a single allele called from the wearer. Then for the swabbings for the touch DNA samples, only one sample yielded a usable DNA profile and it had one allele called for the wearer. As for the M-Vac® samples for blood, all samples yielded a useable DNA profiles with alleles called for the wearer of the t-shirt ranging from 1 to 26 allele calls. With saliva samples collected with the M-Vac®, only one profile was obtained, and it was a partial profile, 16 out of 40 alleles, and it did not have any alleles attributed to the wearer. For the semen samples and the M-Vac® collection method, all samples

gave usable profiles and none of them had alleles from the wearer. Then for the touch DNA samples with the M-Vac®, only one profile was obtained, and it was a partial profile that had 5 out of 40 alleles called. With this one profile, a full profile was obtained from the wearer. Lastly, from all the substrate controls collected from all the t-shirts, ten were blank, and for the 5 that did have alleles from the wearer, the number of alleles called ranged from 1 to 36. The results for the electropherograms can also be found in appendix E.

Just like with the DNA quantity data, unworn and worn t-shirts were compared for the different sample types and the different collection methods that actually yielded a profile that could be interpreted. When comparing the cutting samples with blood, all the unworn and worn t-shirts samples yielded full DNA profiles. For the blood samples collected with the M-Vac®, only 2 of the 3 unworn samples yielded a full profile, whereas the worn samples all yielded full profiles. As for the substrate control samples from both unworn and worn t-shirts that had blood on them, they did not have any full profiles. For the cutting samples with saliva, the worn t-shirts all yielded full profiles and only 3 of the 5 samples for the unworn t-shirts yielded a full profile. When looking at the results from the M-Vac® samples for saliva, only 1 of 3 samples for the unworn samples yielded a full profile and none of the worn samples yielded a full profile. Then for the substrate control samples for the saliva, neither the substrate controls from the unworn or worn yielded a full profile. With the semen samples collected via cuttings, all of the unworn and worn samples yielded full profiles. The same results were seen in the M-Vac® samples for semen for the unworn and worn samples. Also, as with the other results for blood and saliva with the substrate control samples, the substrate control sample for semen for the unworn and worn samples did not yield a full profile. Lastly, when reviewing the touch DNA samples collected via swabs, only 1 of 4 yielded a full DNA profile for both the unworn and worn t-shirts each. For the touch DNA samples collected with M-Vac®, 3 of the 4 samples for the unworn samples yielded a full profile, while the worn sample did not yield any full profile. Then for the substrate control samples for touch DNA, none of the substrate control samples for either unworn or worn samples yielded a full profile. These comparisons did not take into account the wearer DNA from the worn t-shirts or if any partial DNA profiles were obtained either the unworn or worn samples. The results for the electropherograms can also be found in appendix E.

Lastly, when originally designing the experiment, an additional factor was potential contamination between samples collected with the M-Vac® since multiple samples were being placed on the same t-shirt. So, samples were spaced 8 inches away from one another and alternated a male and then female sample next to each other. The DNA quantity data and the results from the unworn t-shirts did not indicate any issues with contamination based on the proximity of the stains. Taking this last part into consideration, it can be determined that cuttings are best for biological fluids that can be seen, and that the M-Vac® is best when a biological sample cannot be seen, such as touch DNA. Additionally, when analyzing the data for the t-shirts worn for 15 minutes, the results indicate that DNA from the participant that wore a t-shirt had the potential to be amplified and appear in a DNA profile for both cuttings or swab and M-Vac®. So, this study needs to be conducted with a t-shirt worn for a longer duration, such as 12 hours, to determine if wear DNA can mask DNA from an evidence sample, such as blood, saliva, semen, or touch DNA. This will also give a better idea of results from an actual casework-type sample.

The initial part of the preliminary study on the best collection method for touch DNA was conducted by WVU undergraduate Jake Nead. The rest of the preliminary study for the unworn and worn cotton t-shirts was conducted by WVU undergraduate Jake Nead and WVU graduate students Kayla Becks, Emma Combs, Nicholas Haas, Phillip Irion, and Kristen Newland.

4. Methods

4.1 Sample collection

First, the research project was explained, a consent was signed, and participants were assigned a random identification number. Afterwards, either a sample of blood, semen (if the participant was male) and or saliva was collected from a participant above the age of 18. These participants were recruited via one-on-one communication. The blood was collected via finger lance from four different participants after each participant had washed their hands. Lastly, the blood was deposited in a 2 mL collection tubes. Approximately 400 μ L of blood was collected from each participant, totalling approximately 1600 μ L. In order to preserve and prevent the blood from coagulating, 40 μ L of PBS buffer and 40 μ L of 0.5 M EDTA was added to a participant's tube after they had deposited all of their sample. As for the semen samples, a participant was supplied with a 15 mL conical polypropylene tube for depositing their sample. Approximately 1500 μ L of semen was collected from the participant that provided this sample. Saliva was collected from one participant spitting into a collection tube totalling approximately 1500 μ L. Additionally, a reference sample was collected from each participant via a buccal swab. Once collected, all samples were labelled with the participant's random identification number and were stored in a -18°C freezer.

4.2 Sample Preparation

Newly purchased white cotton t-shirts were washed with hot water and detergent three times and were dried in a dryer so that the t-shirts were sterile. A participant was given a sterile t-shirt and was instructed to wear the t-shirt for 12 hours consecutively. A t-shirt was given to a participant in a closed brown paper bag and the participant was instructed to place the t-shirt back into the bag when they are done wearing it. All the t-shirts were collected the day of wearing them or the day after they were worn. Within 24 hours of collecting the t-shirts, they were processed for sample preparation. First, t-shirts were cut down the center of the back with sterile scissors and laid flat on a clean lab bench. Then either blood, semen, or saliva from a participant different from the wearer of the t-shirt was dispensed on the front of the t-shirt. The biological fluid was dispensed in 6 different spots on the front of the t-shirt each consisting of 50 μ L each or 300 μ L in total on the t-shirt. These samples were dispensed in the center of a previously marked box that was 4 inches by 4 inches with 2 inches between boxes vertically and 5 inches between boxes horizontally, meaning the boxes were in two columns of three boxes. The left column was for samples that were collected using the M-Vac[®]. These boxes for collection with the M-Vac[®] were outlined with a black sharpie in the four corners of the box. This allowed a zone so that the M-Vac[®] samples would be consistently collected while minimizing the collection of the sharpie dye that could potentially cause issues downstream in sample processing. The right column was for samples collected by cuttings or wet swabs, which were also outlined with a black sharpie in the four corners of the box just as the M-Vac[®] sample column, but also had a smaller 1.5 cm by 1.5 cm sharpie box for dispensing a biological fluid, as seen in figure 13.

Figure 13. Representative Diagram of the t-shirts for the Research Study.

The sharpies were decontaminated with UV light before being used to mark each t-shirt. In addition to this, touch samples were placed on a t-shirt by having a participant, other than the wearer for the t-shirt, grab a t-shirt with both hands for 30 seconds. A participant's left hand grabbed boxes on the left side of the t-shirt and the right hand grabbed boxes on the right side of the t-shirt. Prior to grabbing the t-shirt, the participant was asked to groom their hands by touching their face and or hair and rubbing their hands together for 30 seconds. All biological samples were air dried for 2 hours, while the touch samples were not air dried.

4.3 Collection of M-Vac® and Cutting/Swab samples

First, three substrate control samples were cut from a t-shirt. Two of the substrate control samples were taken from the left and right sleeves, respectfully, and the bottom left side of the t-shirt with a sterilized pair of scissors in approximately 0.5 cm by 0.5 cm squares and placed into a collection tube. Then, the samples from the column on the right side of the t-shirt were cut with sterile scissors from the 1.5 cm by 1.5 cm squares in approximately 0.5 cm by 0.5 cm squares and placed into a collection tube. This was completed for the t-shirts that had blood, saliva, or semen on them. For the touch samples, a wet swab was used instead. The use of a wet swab was determined through a preliminary study showing that swabs were more effective at collecting DNA from a touch sample as opposed to cuttings or scrapping. Lastly, to collect the M-Vac® samples, the instrument was turned on and once the sterile collect solution (SCS) was to pressure, the vacuum was turned on. Then, the SCS was sprayed on a sample location from the right side to the left side of a t-shirt. The sample head collected approximately 25 uL of solution into a sterile collection bottle. The bottle was then closed, labeled appropriately, and set aside until all M-Vac® samples were collected. The sample head and collection bottle were changed to a new sterile one after each sample was collected. The lab bench was also sterilized between t-shirt collections. Once all the samples for the M-Vac® were collected, the instrument was connected to a sterile 0.45 um polyethersulfone (PSE) concentration filter unit. The vacuum was turned on and the collected solution from one sample was poured into the middle of the filter on

the concentration filter unit. The middle of the filter was cut out, perforated with sterile scissors, and then stored in a collection tube. This was completed with a new concentration filter unit for each M-Vac[®] sample. A total of 9 samples were collected per t-shirt for a total of 144 samples (48 M-Vac[®], 48 cuttings/swabs, and 48 substrate controls). In addition, 50 uL control samples from each of the biological fluids used were set aside to be processed as well.

4.4 DNA Extraction and Isolation

DNA was extracted from all the samples utilizing the QIAamp DNA Investigator Kit. The protocol that was utilized for isolating DNA from the cutting/swabs and M-Vac[®] samples was Isolation of Total DNA from Body Fluid Stains where 400 uL of ATL was used instead of 300 uL in order to completely cover samples, not using a QIAshredder column, and eluting with 50 uL of ATE buffer. The same protocol was performed for the control biological fluids samples. All reference samples were extracted utilizing the protocol Isolation of Total DNA from Small Volumes of Blood or Saliva. Approximately 20 uL of DTT was used for any extractions that contained semen as a biological fluid. Also, a reagent blank was processed with every extraction.

4.5 DNA Quantitation

The quantity of DNA for each sample was determined utilizing a 7500 Real-Time PCR instrument and the Quantifiler[™] Trio DNA Quantification Kit following the manufacturer's recommendations. If a sample did not have any indication of being degraded, then the quantity of the small autosomal target was used to either dilute or concentrate a sample to 1 ng/uL. The large autosomal target was used to either concentrate or dilute samples to 1 ng/uL of DNA if it showed any amount of degradation according to the degradation index. If a sample was less than 1 ng/uL, it was concentrated, and if a sample was greater than 1 ng/uL it was diluted.

4.6 DNA Dilution and Concentration

Any samples greater than 1 ng/uL were diluted in TE buffer. Any samples less than 1 ng/uL were concentrated with a Microcon[®] Centrifugal Filter and TB buffer. This concentration step occurred repeatedly until a sample was approximately 15 uL in volume or as close as possible to 1 ng/uL in concentration.

4.7 DNA Amplification

All samples were amplified on a 9700 Gene amp PCR system with the GlobalFiler[™] PCR Amplification Kit following the manufacturer's recommendations.

4.8 Capillary Electrophoresis

All samples were run on a 3500 Genetic Analyzer using the standard protocol from the GlobalFiler[™] PCR Amplification Kit for capillary electrophoresis and the GeneScan[™] 600 LIZ[™] dye Size Standard (v2.0). A 30 kV, 10 second injection time was utilized.

4.9 DNA Quantity Data Interpretation

For analyzing the DNA quantity data from the 7500 real-time PCR instrument, a one-way analysis of variance (ANOVA) was used. The null hypothesis was as follows:

$$H_0: \mu_{\text{M-Vac}^{\text{®}}} = \mu_{\text{Cutting or Swab}} = \mu_{\text{Substrate Control}} \quad (1)$$

This same hypothesis was used for the quantitative data associated with the output for the small autosomal region, the large autosomal region, and the Y-chromosome regions and for each different sample type (i.e. blood, semen, saliva, and touch DNA). The alternative hypothesis was that at least one of the means was different from the other. The α value for ANOVA was 0.05 (i.e. 95% confidence). So, if the p-value was less than 0.05, then the results were statistically significant, therefore the null hypothesis was rejected in favor of the alternate hypothesis and there was a significant difference between means. If the p-value was greater than 0.05, then the results were not statistically significant, therefore the null hypothesis failed to be rejected and there was not a significant difference between means.

Each of the assumptions for ANOVA were addressed either by the experimental design itself or during data analysis. For the assumption concerning independent data, the wearer of the t-shirts and the donors of the biological fluids and or touch DNA never overlapped for each scenario. So, no one contributing biological fluids or touch DNA wore a t-shirt for that biological fluid or touch DNA set and vice versa.

As for the assumption that the dependent variable (i.e. DNA quantity data) was normal, normality was verified qualitatively with Q-Q plots and quantitatively by running the Shapiro-Wilk test. The null hypothesis for the Shapiro-Wilk test was that samples $x_1, \dots, x_n$ came from a normally distributed population. The alternative hypothesis was that samples $x_1, \dots, x_n$ did not come from a normally distributed population. The α value for this test was also 0.05 (i.e. 95% confidence). So, if the p-value was less than 0.05, then the results were statistically significant, therefore the null hypothesis was rejected in favor of the alternate hypothesis and samples $x_1, \dots, x_n$ did not come from a normally distributed population. If the p-value was greater than 0.05, then the results are not statistically significant, therefore the null hypothesis failed to be rejected and samples $x_1, \dots, x_n$ came from a normally distributed population.

The assumption on homogeneity of variances was tested qualitatively with a Residual versus Fitted plot and quantitatively with a Brown-Forsythe test. The Brown-Forsythe Test used deviation from the group median and the null hypothesis was that the population variances were equal to each other. The alternative hypothesis was that the population variances were not equal to each other. The α value for this test was also 0.05. So, if the p-value was less than 0.05, then the results were statistically significant, therefore the null hypothesis was rejected in favor of the alternate hypothesis and the population variances were not equal to one another. If the p-value was greater than 0.05, then the results were not statistically significant, therefore the null hypothesis failed to be rejected and the population variables were equal to one another. If the variances were not equal based on the results of the Brown-Forsythe test, then a Welch's ANOVA was performed.

Welch's ANOVA worked the same way as regular ANOVA with the same null and alternative hypotheses, however, what makes this different is that it could violate the assumption on variance with no penalty and the sampling size per population (i.e. collection method) could be unequal if necessary. Additionally, Welch's ANOVA is determining if there is a difference in population medians as opposed to population means. So, with an α value of 0.05 (i.e. 95% confidence), if the p-value was less than 0.05, then the results were statistically significant, therefore the null hypothesis was rejected in favor of the alternate hypothesis and there was a significant difference between means. If the p-value was greater than 0.05, then the results are not statistically significant, therefore the null hypothesis failed to be rejected and there was not a significant difference between means.

If the results were statistically significant for ANOVA, then a post-hoc test was performed to determine which groups were significantly different from one another. Which post-hoc being performed depended on the outcome of the assumption on variance. If the assumption on variance was not met, then a pairwise t-tests with the Benjamini-Hochberg adjustment was used, but if the assumption was met, then Tukey's Honestly Significant Differences (HSD) post-hoc test was used. For the pairwise t-tests with the Benjamini-Hochberg adjustment, its pairwise comparison also used an α value 0.05. The null hypothesis was that two means being compared were the same and the alternative hypothesis was that the two means being compared were different. So, if the p-value was less than 0.05, then the results were statistically significant, therefore the null hypothesis was rejected in favor of the alternate hypothesis and there was a significant difference between the two means. If the p-value was greater than 0.05, then the results were not statistically significant, therefore the null hypothesis failed to be rejected and there was not a significant difference between the two means.

Finally, for Tukey's Honest Significant Difference (HSD) post-hoc test, it is also a pairwise comparison. Just like every other comparison, the α value was 0.05. The null hypothesis was that the two means being compared were the same and the alternative hypothesis was that the two means being compared were different. So, if the p-value was less than 0.05, then the results were statistically significant, therefore the null hypothesis was rejected in favor of the alternate hypothesis and there was a significant difference between the two means. If the p-value was greater than 0.05, then the results are not statistically significant, therefore the null hypothesis failed to be rejected and there was not a significant difference between the two means.

All these of analyses were coded into R and the source code can be found in appendix 8.1.

4.10 Electropherogram Interpretation and Statistical Analysis

For interpreting the electropherograms, GeneMapper ID-X, Version 1.4, was used. An analytical threshold for all dyes was set to 250 relative fluorescent units (RFUs) other than the size standard, which was set to 400 RFUs. Electropherograms were interpreted for presence or absence of alleles associated with a participant's biological fluid/touch DNA with respect to the presence or absence of the alleles associated with a participant that wore the t-shirt. Control sample electropherograms of the biological fluids were used to determine how many alleles called were related to the biological fluids. As for determining how many alleles called were related to the participant who wore the t-shirt, reference electropherograms were used. This was also performed for the touch DNA samples.

When reviewing allele calls, if one of the alleles called for a heterozygous locus could have been attributed to both the biological sample/touch DNA and the wearer DNA, then a peak height ratio of 60% was used to classify the allele. If the ratio was 60% or greater, it was associated with either the biological fluid/touch DNA or the wearer DNA. However, it was less than 60%, then it was associated with both the biological fluid/touch DNA and the wearer DNA. This was used in scenarios where either two or three alleles were called for a locus, but not when four alleles were called. For a situation when both allele calls were overlapping between the samples at a locus, the sum of the peak heights at that locus were compared to the sum of other peak heights in the same dye channel to determine if possible allelic overlap was occurring. If there was no drastic change between loci, then it was concluded that there was no allelic overlap. However, if there was a drastic change, then allelic overlap was concluded.

Additionally, the electropherogram was also reviewed to see which allele calls, either the biological sample/touch DNA or the wearer DNA, had more of a presence in the electropherogram. The one with more of a presence, or more individualizing allele calls, in the other loci of a sample was what sample the allele call was attributed to. So for example, if there was an allele call that could be associated to the sample DNA or the wearer DNA in one locus and all the other loci in a dye channel were associated with wearer DNA, then the uncertain allele calls would be associated with the wearer and not the sample DNA. The same process occurred if there was allelic overlap and the locus had a homozygous peak. The positive control, negative control, reagent blanks from DNA extraction, and the allelic ladders run were analyzed for quality control. Last, the substrate control samples were analyzed to determine if the background DNA from the participant who wore the t-shirt yielded a full individualizing profile. Any common artifacts seen in electropherograms and contamination were noted for the locus they occurred in for all sample analyzed.

5. Results

5.1 DNA Quantity

5.1.1 Controls, Source samples, and Reference Samples

For the negative control and the reagent blank for the quantitation step for the blood samples, there was either no DNA present or was so low that it would not have been a high enough concentration to amplify to a significant amount to effect downstream interpretation of DNA profiles. As for the positive control, it was at an appropriate concentration based on the kit. The internal positive controls for any of the controls or samples themselves did not indicate any concerns and the standard curves used for these samples for each target quantified were the appropriate values and or concentrations. A few samples showed very minor degradation, so the large autosomal concentration value was used for dilution or concentration as opposed to the small autosomal concentration value. The same can be said for the quantitation runs for the saliva, semen, and touch DNA samples, which also included the source samples for any biological fluids used for this study and any reference samples from participants.

5.1.2 Blood Samples

For the DNA quantity results for the large autosomal target for the Quantifiler™ Trio DNA Quantification Kit for the blood samples, the M-Vac® samples averaged 0.249 ± 0.129 ng/uL, the cutting samples averaged 0.998 ± 0.414 ng/uL, and the substrate control samples averaged 0.00225 ± 0.00195 ng/uL. As for the DNA quantity results for the small autosomal target for blood, the M-Vac® samples averaged 0.242 ± 0.114 ng/uL, the cutting samples averaged 0.847 ± 0.354 ng/uL, and the substrate control samples averaged 0.00297 ± 0.00181 ng/uL. Then for the DNA quantity results for the Y chromosome target for blood, the M-Vac® samples averaged 0.112 ± 0.127 ng/uL, the cutting samples averaged 0.360 ± 0.541 ng/uL, and the substrate control samples averaged 0.00305 ± 0.00278 ng/uL. These results can be seen in Figure 14.

Figure 14. Blood Sample's Mean and Standard Deviation for All Targets. Plots left to right: Large Autosomal Target, Small Autosomal Target, Y Chromosome Target. As for the boxes in the plots, from left to right: M-Vac[®] (red), Cutting (green), Substrate Control (blue). The bar in the middle of the boxes indicate the mean value of the data set and the dots represent each value within a data set. The group cutting had the largest quantity and the largest spread for all targets, whereas the substrate control had the smallest quantity and the smallest spread for all targets.

As for the statistical analysis of the blood samples comparing the M-Vac[®], cuttings, and substrate control groups, all p-values for the large autosomal target were significant values (i.e. below 0.05). This indicated that the means of the different groups being compared with ANOVA were different. When checking the assumptions of ANOVA, this also indicated that the data was not normally distributed, based on the normal Q-Q plot and the p-value for the Shapiro-Wilks test, nor had homogenous variance, based on the residual Vs. fitted plot and p-value for the Brown-Forsythe test. Therefore, the assumptions for ANOVA were violated and Welch's ANOVA was conducted instead. Since the p-value for Welch's ANOVA was also less than 0.05, the same conclusion was reached that there was a significant difference between the groups, specifically the group medians instead of the group means. Lastly, when calculating the p-values for the pairwise t-test with the Benjamini-Hochberg adjustment, it was determined that there was a significant difference between each of the groups being compared. The same can be said about the results for the small autosomal target for the blood samples.

When analyzing the results for the Y chromosome target, the p-value for ANOVA was above 0.05, so there were no significant differences between the group means. However, when checking the assumptions for ANOVA, the assumptions for normality and variance were violated based on the results from the normal Q-Q and residual Vs. fitted plots and the p-values for the Shapiro-Wilks test and the Brown-Forsythe test being less than 0.05. So, just as the other two targets for blood, Welch's ANOVA was performed instead. The p-value for Welch's ANOVA was also significant, indicating a difference in group medians. As for the results for the pairwise t-test with the Benjamini-Hochberg adjustment, the only value that was significant was the comparison between the M-Vac[®] and the substrate control. The other two comparisons were not significant. The results for this target and the other two targets can be seen in Table 1. All of the normal Q-Q plots and residual Vs. fitted plots can be found in appendix 8.2.

Table 1. Overview of Blood Sample's P-Values for the Different Targets. This table contains all the p-values for all statistical tests conducted on each target for the blood samples. All of the p-values were significant (less than 0.05) in this table other than the p-values for blood Y

chromosome target's ANOVA and the pairwise t-tests with the Benjamini-Hochberg adjustment for M-Vac[®] Vs. cutting and substrate control Vs. cutting.

	Blood Large	Blood Small	Blood Y
ANOVA	7.15e-11	3.53e-10	0.0536
Brown-Forsythe Test	1.906e-05	0.0004277	0.04356
Welch's ANOVA	1.645e-07	1.068e-07	0.01482
Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac[®] Vs. Cutting)	4.4e-05	7.6e-05	0.211
Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac[®] Vs. Substrate Control)	4.4e-05	2.3e-05	0.038
Pairwise t-tests with the Benjamini-Hochberg adjustment (Substrate Control Vs. Cutting)	1.3e-05	1.4e-05	0.124
Shapiro-Wilk test	0.0006726	0.0001054	0.000114

5.1.3 Saliva Samples

For the DNA quantity results for the large autosomal target for the Quantifiler™ Trio DNA Quantification Kit for the saliva samples, the M-Vac[®] samples averaged 0.215 ± 0.0582 ng/uL, the cutting samples averaged 0.655 ± 0.305 ng/uL, and the substrate control samples averaged 0.00180 ± 0.00108 ng/uL. As for the DNA quantity results for the small autosomal target for saliva, the M-Vac[®] samples averaged 0.199 ± 0.0522 ng/uL, the cutting samples averaged 0.575 ± 0.246 ng/uL, and the substrate control samples averaged 0.00206 ± 0.00173 ng/uL. Then for the DNA quantity results for the Y chromosome target for saliva, the M-Vac[®] samples averaged 0.0231 ± 0.0288 ng/uL, the cutting samples averaged 0.000997 ± 0.000653 ng/uL, and the substrate control samples averaged 0.00117 ± 0.000793 ng/uL. These results can be seen in Figure 15.

Figure 15. Saliva Sample's Mean and Standard Deviation for All Targets. Plots left to right: Large Autosomal Target, Small Autosomal Target, Y Chromosome Target. As for the boxes in the plots, from left to right: M-Vac[®] (red), Cutting (green), Substrate Control (blue). The bar in the middle of the boxes indicate the mean value of the data set and the dots represent each value within a data set. The group cutting had the largest quantity in all targets other than the Y chromosome target, which was M-Vac[®]. The same can be said about the spread for all targets.

The substrate control had the smallest quantity and the smallest spread for all targets other than the Y chromosome target, which was also the cutting group.

As for the statistical analysis of the saliva samples comparing the M-Vac[®], cuttings, and substrate control groups, all p-values for the large autosomal target were significant values (i.e. below 0.05). This indicated that the means of the different groups being compared with ANOVA were different. When checking the assumptions of ANOVA, this also indicated that the data was not normally distributed, based on the normal Q-Q plot and the p-value for the Shapiro-Wilks test, nor had homogenous variance, based on the residual Vs. fitted plot and p-value for the Brown-Forsythe test. Therefore, the assumptions for ANOVA were violated and Welch's ANOVA was conducted instead. Since the p-value for Welch's ANOVA was also less than 0.05, the same conclusion was reached that there was a significant difference between the groups, specifically the group medians instead of the group means. Lastly, when calculating the p-values for the pairwise t-test with the Benjamini-Hochberg adjustment, it was determined that there was a significant difference between each of the groups being compared. The same can be said about the results for the small autosomal target for the saliva samples and the Y chromosome target with one exception. This exception was the result for the pairwise t-test with the Benjamini-Hochberg adjustment for comparing the substrate control and the cutting groups. The p-value for this comparison was not significantly different. The results for these targets can be seen in Table 2. All of the normal Q-Q plots and residual Vs. fitted plots can be found in appendix 8.2.

Table 2. Overview of Saliva sample's P-Values for the Different Targets. This table contains all the p-values for all statistical tests conducted on each target for the saliva samples. All of the p-values were significant (less than 0.05) in this table other than the p-values for saliva Y chromosome target's pairwise t-tests with the Benjamini-Hochberg adjustment for the substrate control Vs. cutting.

	Saliva Large	Saliva Small	Saliva Y
ANOVA	5.8e-08	4.11e-07	0.033
Brown-Forsythe Test	0.0001112	7.979e-05	0.003935
Welch's ANOVA	2.196e-09	1.189e-09	0.06822
Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac[®] Vs. Cutting)	0.00038	0.00023	0.034
Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac[®] Vs. Substrate Control)	1.9e-07	1.4e-07	0.034
Pairwise t-tests with the Benjamini-Hochberg adjustment (Substrate Control Vs. Cutting)	2.0e-05	9.0e-06	0.683
Shapiro-Wilk test	0.0001286	0.01426	2.987e-05

5.1.4 Semen Samples

For the DNA quantity results for the large autosomal target for the Quantifiler™ Trio DNA Quantification Kit for the semen samples, the M-Vac[®] samples averaged 1.55 ± 0.655 ng/uL, the cutting samples averaged 8.86 ± 4.17 ng/uL, and the substrate control samples averaged 0.003308 ± 0.00476 ng/uL. As for the DNA quantity results for the small autosomal target for semen, the M-Vac[®] samples averaged 1.39 ± 0.593 ng/uL, the cutting samples averaged 8.11 ± 3.95 ng/uL, and the substrate control samples averaged 0.00325 ± 0.00413 ng/uL. Then for

the DNA quantity results for the Y chromosome target for semen, the M-Vac[®] samples averaged 1.38 ± 0.575 ng/uL, the cutting samples averaged 8.00 ± 3.82 ng/uL, and the substrate control samples averaged 0.00360 ± 0.00456 ng/uL. These results can be seen in Figure 16.

Figure 16. Semen Sample's Mean and Standard Deviation for all targets. Plots left to right: Large Autosomal Target, Small Autosomal Target, Y Chromosome Target. As for the boxes in the plots, from left to right: M-Vac[®] (red), Cutting (green), Substrate Control (blue). The bar in the middle of the boxes indicates the mean value of the data set and the dots represent each value within a data set. The group cutting had the largest quantity and the largest spread for all targets, whereas the substrate control had the smallest quantity and the smallest spread for all targets.

As for the statistical analysis of the semen samples comparing the M-Vac[®], cuttings, and substrate control groups, all p-values for the large autosomal target were significant values (i.e. below 0.05). This indicated that the means of the different groups being compared with ANOVA were different. When checking the assumptions of ANOVA, this also indicated that the data was not normally distributed, based on the normal Q-Q plot and the p-value for the Shapiro-Wilks test, nor had homogenous variance, based on the residual Vs. fitted plot and p-value for the Brown-Forsythe test. Therefore, the assumptions for ANOVA were violated and Welch's ANOVA was conducted instead. Since the p-value for Welch's ANOVA was also less than 0.05, the same conclusion was reached that there was a significant difference between the groups, specifically the group medians instead of the group means. Lastly, when calculating the p-values for the pairwise t-test with the Benjamini-Hochberg adjustment, it was determined that there was a significant difference between each of the groups being compared. The same can be said about the results for the small autosomal target and the Y chromosome target for the semen samples. The results for these targets can be seen in Table 3. All of the normal Q-Q plots and residual Vs. fitted plots can be found in appendix 8.2.

Table 3. Overview of Semen Sample's P-Values for the Different Targets. This table contains all the p-values for all statistical tests conducted on each target for the semen samples. All of the p-values were significant (less than 0.05) in this table.

	Semen Large	Semen Small	Semen Y
ANOVA	3.58e-10	7.56e-10	3.01e-09
Brown-Forsythe Test	3.389e-07	1.022e-06	1.849e-06
Welch's ANOVA	1.109e-07	1.458e-07	1.111e-07

Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac® Vs. Cutting)	7.4e-05	9.6e-05	8.1e-05
Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac® Vs. Substrate Control)	1.6e-05	1.8e-05	1.4e-05
Pairwise t-tests with the Benjamini-Hochberg adjustment (Substrate Control Vs. Cutting)	2.2e-05	3.0e-05	2.5e-05
Shapiro-Wilk test	8.88e-05	6.755e-05	0.0003065

5.1.5 Touch DNA Samples

For the DNA quantity results for the large autosomal target for the Quantifiler™ Trio DNA Quantification Kit for the touch DNA samples, the M-Vac® samples averaged 0.0711 ± 0.500 ng/uL, the swabbing samples averaged 0.00309 ± 0.00256 ng/uL, and the substrate control samples averaged 0.00800 ± 0.00982 ng/uL. As for the DNA quantity results for the small autosomal target for the touch DNA samples, the M-Vac® samples averaged 0.0665 ± 0.0397 ng/uL, the swabbing samples averaged 0.00430 ± 0.00330 ng/uL, and the substrate control samples averaged 0.00919 ± 0.0110 ng/uL. Then for the DNA quantity results for the Y chromosome target for the touch DNA samples, the M-Vac® samples averaged 0.0397 ± 0.0588 ng/uL, the swabbing samples averaged 0.00103 ± 0.000781 ng/uL, and the substrate control samples averaged 0.00640 ± 0.0110 ng/uL. These results can be seen in Figure 17.

Figure 17. Touch DNA Sample's Mean and Standard Deviation for all targets. Plots left to right: Large Autosomal Target, Small Autosomal Target, Y Chromosome Target. As for the boxes in the plots, from left to right: M-Vac® (red), Cutting (green), Substrate Control (blue). The bar in the middle of the boxes indicates the mean value of the data set and the dots represent each value within a data set. The group M-Vac® had the largest quantity and the largest spread for all targets, whereas the swabbing group had the smallest quantity and the smallest spread for all targets.

As for the statistical analysis of the touch DNA samples comparing the M-Vac®, swabbing, and substrate control groups, all p-values for the large autosomal target were significant values (i.e. below 0.05) except for one, which was the pairwise t-test with the Benjamini-Hochberg adjustment comparing the substrate control and swabbing groups. This indicated that the means of the different groups being compared with ANOVA were different. When checking the assumptions of ANOVA, this also indicated that the data was not normally distributed, based on the normal Q-Q plot and the p-value for the Shapiro-Wilks test, nor had

homogenous variance, based on the residual Vs. fitted plot and p-value for the Brown-Forsythe test. Therefore, the assumptions for ANOVA were violated and Welch's ANOVA was conducted instead. Since the p-value for Welch's ANOVA was also less than 0.05, the same conclusion was reached that there was a significant difference between the groups, specifically the group medians instead of the group means. Lastly, when calculating the p-values for the pairwise t-test with the Benjamini-Hochberg adjustment, it was determined that there was a significant difference between each of the groups being compared other than the comparison between the substrate control and swabbing groups. The same can be said about the results for the small autosomal target for the touch DNA samples.

When analyzing the results for the Y chromosome target for the touch DNA samples, the p-value for ANOVA was above 0.05, so there was no significant difference between the group means. When checking the assumptions for ANOVA, the assumptions for normality was violated based on the results from the normal Q-Q and the p-values for the Shapiro-Wilks test. However, the assumption on variance was not violated. So, Welch's ANOVA was not performed. Another difference with this analysis as opposed to the other two targets, was that Tukey Honestly Significant Difference (HSD) post-hoc test was conducted instead of the pairwise t-test with the Benjamini-Hochberg adjustment. This was because there was no violation of the assumption on variance. All of the p-values for this post-hoc test were not significant for the comparisons between the different groups. The results for this target and the other two targets can be seen in Table 4. All of the normal Q-Q plots and residual Vs. fitted plots can be found in appendix 8.2.

Table 4. Overview of Touch DNA sample's P-Values for the Different Targets. This table contains all the p-values for all statistical tests conducted on each target for the touch DNA samples. All of the p-values were significant (less than 0.05) in this table other than the p-values for the pairwise t-tests with the Benjamini-Hochberg adjustment for Substrate Control Vs. swabbing for the large and small autosomal targets. As for the Y chromosome targets, none of the p-values were significantly different other than for the Shapiro-Wilk test.

	Touch DNA Large	Touch DNA Small	Touch DNA Y
ANOVA	2.13e-06	2.25e-07	0.0593
Brown-Forsythe Test	0.001527	0.001397	0.09677
Welch's ANOVA	0.0007315	0.0002216	NA
Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac® Vs. Swabbing)	0.0016	0.00054	NA
Pairwise t-tests with the Benjamini-Hochberg adjustment (M-Vac® Vs. Substrate Control)	0.0016	0.00054	NA
Pairwise t-tests with the Benjamini-Hochberg adjustment (Substrate Control Vs. Swabbing)	0.1192	0.15971	NA
Tukey Honest Significant Difference Post-hoc test (M-Vac® Vs. Swabbing)	NA	NA	0.1021103
Tukey Honest Significant Difference Post-hoc test (M-Vac® Vs. Substrate Control)	NA	NA	0.1103890
Tukey Honest Significant Difference Post-hoc test (Substrate Control Vs. Swabbing)	NA	NA	0.9544224
Shapiro-Wilk test	1.852e-06	5.223e-05	1.34e-07

5.2 Electropherogram Interpretation

All electropherograms and profile summary forms can be viewed upon request.

5.2.1 Controls, Source Samples, and Reference Samples

For all the samples run on the 3500 Genetic Analyzer, the reagent blanks from extraction did not have any alleles called in their electropherograms. For the positive control for each run, any time there were discrepancies and or no profile was obtained when one should have been, the plate was reinjected on the 3500 Genetic Analyzer, the samples were remade and rerun, or reamplified when necessary and rerun. Additionally, if the source of the discrepancy was known and was isolated to the control or could be accounted for in other samples, the samples were not rerun or reamplified. The same was performed with any discrepancies or if a negative electropherogram was not obtained for the negative control. Lastly, all allelic ladders for every run did not have any issues or discrepancies. The same can be said for the electropherograms for the biological fluids source samples and the reference samples for participants.

5.2.2 Blood Samples

When reviewing the electropherograms for the blood samples collected by taking a cutting, 9 of the 12 profiles yielded a full profile, while 3 of the 12 yielded a partial profile. The average number of alleles called for the partial profiles was 17 alleles of the 39 or 40 alleles possible. As for the wearer DNA with these samples, none of these yielded a full profile and 2 of the 12 yielded a partial profile with the average number of alleles called being 2 out of the possible 39 or 40 alleles. The other 10 profiles did not indicate any wearer DNA being present in the samples. A summary of the cutting samples can be found below in table 5.

Table 5. Summary Table of Blood Samples Collected using Cuttings. This table is a comparison between samples of blood and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from blood samples collected with the cutting method.

	Sample	Wearer
Number of Full Profiles	9/12	0/12
Number of Partial Profiles	3/12	2/12
Number with no Allele Calls	0/12	10/12
Average Number of Allele Calls for a Partial Profile	$51/3 = 17$	$4/2 = 2$

With the electropherograms for the blood samples collected with the M-Vac®, 10 of the 12 samples yielded a full profile and the other 2 samples were partial profiles with an average of 27 of the 40 alleles present. For the wearer DNA with these samples, 1 yielded a full DNA profile and the other 11 were partial profiles with the average number of allele calls being 26 out of 39, 40, or 43 alleles. A summary of the M-Vac® samples can be found below in table 6.

Table 6. Summary Table of Blood Samples Collected using M-Vac®. This table is a comparison between samples of blood and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from blood samples collected with the M-Vac® method.

	Sample	Wearer
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Number of Full Profiles	10/12	1/12
Number of Partial Profiles	2/12	11/12
Number with no Allele Calls	0/12	0/12
Average Number of Allele Calls for a Partial Profile	$54/2 = 27$	$286/11 = 26$

As for the electropherograms for the substrate control samples for the t-shirt that blood was dispensed on, there were 3 partial profiles that had allele calls that could have potentially been attributed to the blood placed on the shirt with an average of 2 alleles called out of the 39 or 40 possible alleles. The other 8 substrate control samples did not yield any electropherograms related to the blood placed on the t-shirts. As for the wearer DNA, 11 of the electropherograms produced a partial profile with an average of approximately 20 allele calls out of the possible 39, 40, or 43 alleles. There was 1 electropherogram for which the blood sample nor the wearer DNA could be determine. A summary of the substrate control samples can be found below in table 7.

Table 7. Summary Table of the Substrate Control Samples from the Blood Sample T-shirts. This table is a comparison between samples of blood and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from the substrate controls taken from the t-shirts used for the blood samples. One sample was uninterpretable due to allelic ladder contamination.

	Sample	Wearer
Number of Full Profiles	0/12	0/12
Number of Partial Profiles	3/12	11/12
Number with no Allele Calls	9/12	0/12
Number of Uninterpretable Profiles	1/12	1/12
Average Number of Allele Calls for a Partial Profile	$6/3 = 2$	$219/11 = 19.91$

5.2.3 Saliva Samples

When reviewing the electropherograms for the saliva samples collected by taking a cutting, 11 of the 12 profiles yielded a full profile, while only 1 was a partial profile with 32 of the 39 possible alleles present. As for the wearer DNA with these samples, none of these yielded a full profile and 2 of the 12 yielded a partial profile with the average number of alleles called being 2 out of the possible 39 alleles. The other 10 profiles did not indicate any wearer DNA being present in the samples. A summary of the cutting samples can be found below in table 8.

Table 8. Summary Table of Saliva Samples Collected using Cuttings. This table is a comparison between samples of saliva and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from saliva samples collected with the cutting method.

	Sample	Wearer
Number of Full Profiles	11/12	0/12
Number of Partial Profiles	1/12	2/12
Number with no Allele Calls	0/12	10/12
Average Number of Allele Calls for a Partial Profile	$32/1 = 32$	$4/2 = 2$

With the electropherograms for the saliva samples collected with the M-Vac®, all 12 samples yielded a full profile. For the wearer DNA with these samples, 1 yielded a full DNA

profile and the other 9 were partial profiles with the average number of allele calls being approximately 23 out of the 39, 40, or 43 alleles. As for the other 2 profiles, they did not indicate an allele calls from the wearer DNA. A summary of the M-Vac® samples can be found below in table 9.

Table 9. Summary Table of Saliva Samples Collected using M-Vac®. This table is a comparison between samples of saliva and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from saliva samples collected with the M-Vac® method.

	Sample	Wearer
Number of Full Profiles	12/12	1/12
Number of Partial Profiles	0/12	9/12
Number with no Allele Calls	0/12	2/12
Average Number of Allele Calls for a Partial Profile	NA	209/9 = 23.22

As for the electropherograms for the substrate control samples for the t-shirt that saliva was dispensed on, there were 2 partial profiles that had allele calls that could have potentially been attributed to the saliva placed on the shirt. One profile had 1 of the possible 39 alleles called and the other profile had approximately 2 of 39 possible alleles called. All the other substrate control samples did not yield any electropherograms related to the saliva placed on the t-shirts. As for the wearer DNA, 9 of the electropherograms produced a partial profile with an approximate average of 19 allele calls out of the possible 39, 40, or 43 alleles. The other was 3 electropherogram did not have any indication of wearer DNA being in the samples. A summary of the substrate control samples can be found below in table 10.

Table 10. Summary Table of the Substrate Control Samples from the Saliva Sample T-shirts. This table is a comparison between samples of saliva and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from the substrate controls taken from the t-shirts used for the saliva samples.

	Sample	Wearer
Number of Full Profiles	0/12	0/12
Number of Partial Profiles	2/12	9/12
Number with no Allele Calls	10/12	3/12
Average Number of Allele Calls for a Partial Profile	3/2 = 1.5	168/9 = 18.67

5.2.4 Semen Samples

When reviewing the electropherograms for the semen samples collected by taking a cutting, 2 of the 12 profiles yielded a full profile and 10 were partial profile with an average of approximately 35 of the 40 possible alleles present. As for the wearer DNA with these samples, none of these yielded any profiles with allele calls related to participants that wore t-shirts. A summary of the cutting samples can be found below in table 11.

Table 11. Summary Table of Semen Samples Collected using Cuttings. This table is a comparison between samples of semen and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from semen samples collected with the cutting method.

	Sample	Wearer
Number of Full Profiles	2/12	0/12
Number of Partial Profiles	10/12	0/12
Number with no Allele Calls	0/12	12/12
Average Number of Allele Calls for a Partial Profile	347/10 = 34.7	NA

With the electropherograms for the semen samples collected with the M-Vac®, 7 of the 12 samples yielded a full profile. The other 5 samples were partial profiles that had an average of approximately 36 of 40 potential alleles called. For the wearer DNA with these samples, 5 were partial profiles with the average number of allele calls being approximately 8 out of the 40 alleles. As for the other 7 profiles, they did not indicate an allele calls from the wearer DNA. A summary of the M-Vac® samples can be found below in table 12.

Table 12. Summary Table of Semen Samples Collected using M-Vac®. This table is a comparison between samples of semen and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from semen samples collected with the M-Vac® method.

	Sample	Wearer
Number of Full Profiles	7/12	0/12
Number of Partial Profiles	5/12	5/12
Number with no Allele Calls	0/12	7/12
Average Number of Allele Calls for a Partial Profile	179/5 = 35.8	39/5 = 7.8

As for the electropherograms for the substrate control samples for the t-shirt that semen was dispensed on, there were 2 full profiles that had allele calls that could have potentially been attributed to the saliva placed on the shirt. Five of the profiles were partial profile with an average of approximately 6 of the possible 40 alleles called. Two of the other profile did not yield any electropherograms related to the semen placed on the t-shirts. As for the wearer DNA, 6 of the electropherograms produced a partial profile with an average of approximately 9 allele calls out of the possible 39 or 40 alleles. The other was 3 electropherogram did not have any indication of wearer DNA being in the samples. The last 3 samples did not yield an electropherogram for which the semen sample nor the wearer DNA could be determine. A summary of the substrate control samples can be found below in table 13.

Table 13. Summary Table of the Substrate Control Samples from the Semen Sample T-shirts. This table is a comparison between samples of semen and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from the substrate controls taken from the t-shirts used for the semen samples. One the samples was uninterpretable since a profile could not be obtained for it and the other two had allelic ladder contamination.

	Sample	Wearer
Number of Full Profiles	2/12	0/12
Number of Partial Profiles	5/12	6/12
Number with no Allele Calls	2/12	3/12
Number of Uninterpretable Profiles	3/12	3/12
Average Number of Allele Calls for a Partial Profile	29/5 = 5.8	53/6 = 8.83

5.2.5 Touch DNA Samples

When reviewing the electropherograms for the touch DNA samples collected by a wet swab, 1 of the 12 profiles yielded a full profile and 6 were partial profile with an average of approximately 12 of the 37 possible alleles present. The other 4 electropherograms did not have any indication of the touch DNA from the participant for these samples. As for the wearer DNA with these samples, 10 of the samples yielded partial profiles with an average of approximately 17 alleles called from the possible 39, 40 or 43. The other electropherogram did not have an indication of wearer DNA being in the sample. There was 1 electropherogram for which the touch DNA sample nor the wearer DNA could be determine. A summary of the cutting samples can be found below in table 14.

Table 14. Summary Table of Touch DNA Samples Collected using Wet Swabs. This table is a comparison between samples of touch DNA and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from touch DNA samples collected with the wet swab method. One sample was uninterpretable due to allelic ladder contamination.

	Sample	Wearer
Number of Full Profiles	1/12	0/12
Number of Partial Profiles	6/12	10/12
Number with no Allele Calls	4/12	1/12
Number of Uninterpretable Profiles	1/12	1/12
Average Number of Allele Calls for a Partial Profile	70 /6 = 11.67	172/10 = 17.2

With the electropherograms for the touch DNA samples collected with the M-Vac®, all 12 samples yielded partial profiles with an average of approximately 20 of the 37 possible alleles present. As for the wearer DNA with these samples, all 12 yielded full profiles. A summary of the M-Vac® samples can be found below in table 15.

Table 15. Summary Table of Touch DNA Samples Collected using M-Vac®. This table is a comparison between samples of touch DNA and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from touch DNA samples collected with the M-Vac® method.

	Sample	Wearer
Number of Full Profiles	0/12	12/12
Number of Partial Profiles	12/12	0/12
Number with no Allele Calls	0/12	0/12
Average Number of Allele Calls for a Partial Profile	242/12 = 20.17	NA

As for the electropherograms for the substrate control samples for the t-shirt that touch DNA was placed on, there were 4 partial profile with an average of about 7 of the possible 37 alleles called. Seven of the other profile did not yield any electropherograms related to the touch DNA placed on the t-shirts. As for the wearer DNA, 5 of the electropherograms produced a full profile and 5 a partial profile with an average of about 23 allele calls out of the possible 39, 40, or 43 alleles. The other electropherogram did not have any indication of wearer DNA being in the samples. There was 1 electropherogram for which the touch DNA sample nor the wearer

DNA could be determine. A summary of the substrate control samples can be found below in table 16.

Table 16. Summary Table of the Substrate Control Samples from the Touch DNA Sample T-shirts. This table is a comparison between samples of touch DNA and the wearer DNA for the number of full DNA profiles and the number of partial profiles with the average number of allele calls obtained from the substrate controls taken from the t-shirts used for the touch DNA samples. One sample was uninterpretable due to allelic ladder contamination.

	Sample	Wearer
Number of Full Profiles	0/12	5/12
Number of Partial Profiles	4/12	5/12
Number with no Allele Calls	7/12	1/12
Number of Uninterpretable Profiles	1/12	1/12
Average Number of Allele Calls for a Partial Profile	26/4 = 6.5	113/5 = 22.6

6. Discussion

6.1 DNA Quantity

One of the goals for this study was to determine which method, M-Vac[®] vs. traditional method, collected more DNA from an evidence sample, specifically a t-shirt worn for 12 hours consecutively. With samples of biological fluids, the M-Vac[®] was compared to a cutting, and with touch DNA, the M-Vac[®] was compared to a wet swab.

6.1.1 Blood Samples

Based on the mean values of DNA quantitated for the blood samples, cuttings yielded a higher quantity of DNA as opposed to the M-Vac[®], especially for the large and small autosomal targets which are used for either diluting or concentrating DNA samples for amplification; meaning that the cutting method is more efficient at collecting DNA than the M-vac[®] method for blood stains. For the large autosomal target, the cutting sample collected 4 times more DNA than the M-Vac[®]. As for the small autosomal target, the cutting samples collected about 3.5 times the amount of DNA as opposed to the M-Vac[®] sample. Then for the Y chromosome target, the cutting samples collected about 3.2 times more DNA than the M-Vac[®] samples. These conclusions were supported by the statistical analyses performed for this study. According to the pairwise t-test with the Benjamini-Hochberg adjustments, there was a significant difference in the amount of DNA collected when comparing the M-Vac[®] and the cutting method for the large and small autosomal targets. While there was not a difference between these two methods for the Y chromosome target. This can be explained by the fact that only 3 of the 8 participants for this part of this study were male, two of which wore t-shirts and one who donated blood. For the male participant's blood that was used, it was not used on a t-shirt that a male participant wore, However, neither collection methods were particularly close to the amount of DNA from the blood placed on the t-shirts to begin with, which was approximately 13.13 ng/uL for the large autosomal target, 12.76 ng/uL for the small autosomal target, and 4.31 ng/uL for the Y chromosome target. For the large and small autosomal targets, the M-Vac[®] collected about 1.9% of the average blood source sample, and for the Y chromosome target, the M-Vac[®] collected approximately 2.6% of the average blood source sample. As for the cutting samples, for the large autosomal target, the cutting method collected about 7.6%, for the small autosomal target the cutting method collected approximately 6.64%, and for the Y chromosome the cutting method collected around 8.35% of the average blood source sample place on the t-shirts.

Since there were DNA quantities for the substrate control samples, though they were extremely low, they did indicate the potential for wearer DNA to be collected along with the blood samples from using either of these collection methods and then could be subsequently amplified. For the Large autosomal target, the wearer DNA could have been up to an average of 0.9% of the M-Vac® samples and up to an average of 0.23% of the cutting samples. The statement “up to” is being used in this when comparing the DNA quantity from the samples themselves to the Substrate controls. Theoretically, the substrate control should be the wearer only, so it is hypothesized that “up to” the quantity of the substrate control (i.e. wearer DNA) could be a part of the actual samples themselves when being quantitated. So, the DNA quantity for the large autosomal target for blood was an averaged 0.249 ± 0.129 ng/uL and the substrate control samples averaged 0.00225 ± 0.00195 ng/uL. Therefore 0.00225 ng/uL of the 0.249 ng/uL, or 0.9%, of blood sample could be from the wearer DNA. As for the small autosomal target, the wearer DNA could have been up to an average of 1.23% of the M-Vac® samples and up to an average of 0.35% of the cutting samples. Then for the Y chromosome target, the wearer DNA could have been up to an average of 2.72% of the M-Vac® samples and up to an average of 0.85% of the cutting samples. This did help to explain some of the results of the electropherogram that are discussed later in this section.

6.1.2 Saliva Samples

Based on the mean values of DNA quantitated for the saliva samples, cuttings yielded a higher quantity of DNA as opposed to the M-Vac®, especially for the large and small autosomal targets which are used for either diluting for concentrating DNA samples for amplification; meaning that the cutting method is more efficient at collecting DNA than the M-vac® method for saliva stains. For the large autosomal target, the cutting sample collected 3.05 times more DNA than the M-Vac®. As for the small autosomal target, the cutting samples collected about 2.89 times the amount of DNA as opposed to the M-Vac® sample. The Y chromosome target was different, and the M-Vac® collected more DNA than the cutting method. The M-Vac® samples collected about 23 times more DNA than the cutting samples. This was likely because the saliva samples for all t-shirts was female and two of the participants that wore t-shirts were male. So, cuttings are still considered to collect more DNA than the M-Vac® method. These conclusions were supported by the statistical analyses performed for this study. According to the pairwise t-test with the Benjamini-Hochberg adjustments, there was a significant difference in the amount of DNA collected when comparing the M-Vac® and the cutting method for all targets. However, neither collection methods were particularly close to the amount of DNA from the saliva placed on the t-shirts to begin with, which was 4.50 ng/uL for the large autosomal target and 3.89 ng/uL for the small autosomal target. For the large autosomal target, the M-Vac® collected about 4.8% of the saliva source sample, and for the small autosomal target, the M-Vac® collected approximately 5.11% of the control saliva source sample. As for the cutting samples, for the large autosomal target about 14.6% was collected and for the small autosomal target approximately 14.78% of the saliva source sample collected.

Since there were DNA quantities for the substrate control samples and for the Y chromosome targets for the M-Vac® and cutting samples, it indicated the potential for wearer DNA to be collected along with the saliva samples from using either of these collection methods and then could be subsequently amplified. For the large autosomal target, the wearer DNA could have been up to an average of 0.84% of the M-Vac® samples and up to an average of 0.27% of the cutting samples. As for the small autosomal target, the wearer DNA could have been up to an

average of 1.04% of the M-Vac[®] samples and up to an average of 0.36% of the cutting samples. Then for the Y chromosome target, the wearer DNA did contribute 0.0231 ng/uL of DNA to the M-Vac[®] samples and 0.000997 ng/uL of DNA to the cutting samples since the saliva sample used was from a female individual. This did help to explain some of the results of the electropherogram that are discussed later in this section.

6.1.3 Semen Samples

Based on the mean values of DNA quantitated for the semen samples, cuttings yielded a higher quantity of DNA as opposed to the M-Vac[®], especially for the large and small autosomal targets which are used for either diluting for concentrating DNA samples for amplification; meaning that the cutting method is more efficient at collecting DNA than the M-Vac[®] method for semen stains. For the large autosomal target, the cutting samples collected 5.72 times more DNA than the M-Vac[®]. As for the small autosomal target, the cutting samples collected about 5.83 times the amount of DNA as opposed to the M-Vac[®] sample. Then for the Y chromosome target, the cutting samples collected about 5.8 times more DNA than the M-Vac[®] samples. These conclusions were supported by the statistical analyses performed for this study. According to the pairwise t-test with the Benjamini-Hochberg adjustments, there was a significant difference in the amount of DNA collected when comparing the M-Vac[®] and the cutting method for all the targets. However, neither collection methods were particularly close to the amount of DNA from the semen placed on the t-shirts to begin with, which was 17.62 ng/uL for the large autosomal target, 17.02 ng/uL for the small autosomal target, and 19.13 ng/uL for the Y chromosome target. For the large autosomal target, the M-Vac[®] collected about 8.8% of the semen source sample, for the small autosomal target the M-Vac[®] collected approximately 8.17% of the semen source sample, and for the Y chromosome target the M-Vac[®] collected approximately 7.21% of the semen source sample. As for the cutting samples, 50.28% of the semen source sample was collected for the large autosomal target, for the small autosomal target approximately 47.65% of the semen source sample was collected, and for the Y chromosome target approximately 41.82% of the semen source sample was collected.

Since there were DNA quantities for the substrate control samples, though they were extremely low, they did indicate the potential for wearer DNA to be collected along with the semen samples from using either of these collection methods and then could be subsequently amplified. For the large autosomal target, the wearer DNA could have been up to an average of 0.21% of the M-Vac[®] samples and up to an average of 0.037% of the cutting samples. As for the small autosomal target, the wearer DNA could have been up to an average of 0.23% of the M-Vac[®] samples and up to an average of 0.04% of the cutting samples. Then for the Y chromosome target, the wearer DNA could have been up to an average of 0.33% of the M-Vac[®] samples and up to an average of 0.045% of the cutting samples. This did help to explain some of the results of the electropherogram that are discussed later in this section.

6.1.4 Touch DNA Samples

Based on the mean values of DNA quantitated for the touch DNA samples, the M-Vac[®] yielded a higher quantity of DNA as opposed to the wet swab samples, especially for the large and small autosomal targets which are used for either diluting for concentrating DNA samples for amplification; meaning that the M-Vac[®] method is more efficient at collecting DNA than the wet swab method for touch DNA. For the large autosomal target, the M-Vac[®] samples collected 23 times more DNA than the wet swabs. As for the small autosomal target, the M-Vac[®] samples

collected about 15.47 times the amount of DNA as opposed to the wet swab samples. Also, Y chromosome target showed that M-Vac® collected more DNA than the wet swab method. The M-Vac® samples collected about 38.54 times more DNA than the wet swab samples. This was likely because there was so much more wearer DNA from the shirt as opposed to the touch DNA samples, which was a participant grabbing a t-shirt for 30 seconds aggressively. These conclusions were supported by the statistical analyses performed for this study for the large and small autosomal targets. According to the pairwise t-test with the Benjamini-Hochberg adjustments, there was a significant difference in the amount of DNA collected when comparing the M-Vac® and the wet swab method for those targets. However, there was not a difference between the M-Vac® and wet swab method for the Y chromosome region. This might have occurred since two of the participants who wore t-shirts were male and the touch DNA only came from a female individual for all t-shirts. The amount of touch DNA added to t-shirts by a participant could not be quantitated, and therefore the quantity values for the t-shirt could not really be compared to a sample control to determine how much DNA was not collected from either of these two collection methods.

Since there were DNA quantities for the substrate control samples and for the Y chromosome targets for the M-Vac® and wet swab samples, it indicated the potential for wearer DNA to be collected along with the touch DNA samples from using either of these collection methods and then could be subsequently amplified. For the Large autosomal target, the wearer DNA could have been up to an average of 11.25% of the M-Vac® samples and potentially all of the wet swab samples since the quantity for the large autosomal target for the substrate control was more than the quantity of the large autosomal target for the wet swab samples. As for the small autosomal target, the wearer DNA could have been up to an average of 13.82% of the M-Vac® samples and again potentially all of the wet swab samples since the quantity for the small autosomal target for the substrate control was more than the quantity of the small autosomal target for the wet swab samples.. Then for the Y chromosome target, the wearer DNA could have been up to an average of 16.12% of the M-Vac® samples and yet again potentially all of the wet swab samples since the quantity for the Y chromosome target for the substrate control was more than the quantity of the Y chromosome target for the wet swab samples. This did help to explain some of the results of the electropherogram that are discussed later in this section.

6.1.5 Explanation of Discrepancies and Future Directions

One reason why the DNA quantity collected was low compared to the control biological source sample could be because some of the DNA was still left in solution on the t-shirt itself or left in micro water droplets left in the collection bottle. Another reason for the low quantity for some of the M-Vac® samples could be that some of the DNA collected was cell free DNA that was not caught on concentration filter whereas the control biological source samples and their potential cell free DNA was contained in a single tube when DNA extraction was performed. This was probable based on the previously mention study by Vickara *et al.* (2018) where they showed the cell free DNA is not necessarily caught by the concentration filter. Another possibility to explain this loss of sample for some of the M-Vac® samples could have been due to only a portion of the center of the concentration filter being used for DNA extraction as opposed to the entire filter. Additionally, this can explain some of the discrepancies between this study showing that the traditional method of cuttings was more efficient than the M-Vac® for collecting DNA from biological fluids as other than the other way around, such as from M-Vac® System, Inc., Smolar *et al.* (2014), and McLamb *et al.* (2020). This same procedure of not fully

consuming an evidence sample could be explained the quantity of the DNA from cuttings not being consistent with the biological source samples since only a portion of the biological stain was taken for a cutting. As an explanation as to why only portion of the M-Vac[®] concentration filter and the biological stain is being taken for a cutting, is because it is common practice in forensic DNA laboratories that the whole evidence sample should not be consumed if possible while processing incase an evidence sample needs to be reprocessed by the same laboratory or a different laboratory.¹ Another explanation for the discrepancy with DNA quantity of the samples and the control biological source samples for both the M-Vac[®] and the cutting was QIAshredder columns not being used during DNA extraction.

Some future directions for this would be to process the collection fluid flow through from the M-Vac[®] collection filtration unit to determine if any cell free DNA was lost during this step. Additionally, changes could be made to the extraction process to use the entire M-Vac[®] concentration filter or biological stain cutting with a QIAshredder columns to increase the yield of DNA from a sample.

6.2 Electropherogram Interpretation

The second goal for this study was to determine if wearer DNA is also collected with biological samples of blood, saliva, and semen, and touch DNA samples from a t-shirt worn for 12 hours consecutively using both the M-Vac[®] and traditional methods, cuttings and wet swab, and if so, how it affected on the interpretation of the resulting DNA profiles for these samples.

6.2.1 Blood Samples

The results from the DNA quantity data indicated that cuttings obtained more DNA from blood samples as opposed to the M-Vac[®] and were therefore more efficient. This was not necessarily true when reviewing the DNA profiles for these samples. The twelve cutting samples for blood yielded 9 full profiles and 3 partial profiles with an average allele call of 17, whereas the 12 M-Vac[®] samples for blood yielded 10 full profiles and 2 partial profiles with an average allele call of 27. So, from this data, the M-Vac[®] collection method was better than the cutting method for both the number of full profiles obtained and the number of alleles called for partial profiles.

There was also some evidence from the DNA quantity data that wearer DNA could be amplified with the sample DNA of the blood. This was verified in the M-Vac[®] samples for the blood since 1 full profile from the wearer of a t-shirt was able to be pulled out of 1 of the blood samples. Additionally, for the other 11 blood samples collected by M-Vac[®], partial profiles with an average number of 26 alleles were able to be pulled out of the electropherogram for the wearer of the t-shirts. As for the cutting samples, of the 12 blood samples, 2 partial profiles with an average number of 2 alleles for the wearer DNA were able to be pulled out of the electropherograms. These results for the M-Vac[®] and the cuttings also add to the conclusion that the M-Vac[®] is better than the cutting method if one is trying to determine the wearer of the t-shirt as well as the blood sample on the t-shirt.

As for the substrate control samples, 11 of the 12 samples were partial profiles with an average of 20 alleles for the wearer DNA, which means that there was at least enough DNA from the wearer of the t-shirts to be collected and amplified with both the M-Vac[®] and cutting methods and potentially affect the interpretation of the electropherograms. Ultimately though, the interpretation for these blood samples were not necessarily affected by the wearer DNA also

being amplified with the blood DNA. This was likely because although there was wearer DNA being amplified, it was less than the DNA from the blood samples themselves.

6.2.2 Saliva Samples

The results from the DNA quantity data indicated that cuttings obtained more DNA from saliva samples as opposed to the M-Vac® and were therefore more efficient. This was not necessarily true for these samples either when reviewing the DNA profiles. The twelve cutting samples for saliva yielded 11 full profiles and 1 partial profiles with an average number of 32 allele call, whereas the 12 M-Vac® samples for saliva all yielded full profiles. So, from this data, the M-Vac® collection method was better than the cutting method.

There was also some evidence from the DNA quantity data that wearer DNA could be amplified with the sample DNA of the saliva. This was verified in the M-Vac® samples for the saliva since 1 full profile from the wearer of a t-shirt was able to be pulled out of 1 of the blood sample. Additionally, for 9 of the blood samples collected by M-Vac®, partial profiles with an average number 23 alleles called were able to be pulled out of the electropherogram for the wearer of the t-shirts. As for the cutting samples, of the 12 saliva samples, 2 partial profiles with an average number of 2 alleles for the wearer DNA were able to be pulled out of the electropherograms. These results for the M-Vac® and the cuttings also add to the conclusion that the M-Vac® is better than the cutting method if one is trying to determine the wearer of the t-shirt as well as the saliva sample on the t-shirt.

Then for the substrate control samples, 9 of the 12 samples were partial profiles with an average allele call of 19 for the wearer DNA, which means that there was at least enough DNA from the wearer of the t-shirts to be collected and amplified with both the M-Vac® and cutting methods and potentially affect the interpretation of the electropherograms. Ultimately though, the interpretation for these saliva samples were not necessarily affected by the wearer DNA also being amplified with the saliva DNA. This was likely because although there was wearer DNA being amplified, it was not more than the DNA from the saliva samples themselves.

6.2.3 Semen Samples

The results from the DNA quantity data indicated that cuttings obtained more DNA from semen samples as opposed to the M-Vac® and were therefore more efficient. This was not necessarily true for the semen samples as well when reviewing the DNA profiles. The twelve cutting samples for saliva yielded 2 full profiles and 10 partial profiles with an average number of 35 alleles called, whereas the 12 M-Vac® samples for saliva yielded 7 full profiles and 5 partial profiles with an average of 36 allele calls. So, from this data, the M-Vac® collection method was better than the cutting method.

There was also some evidence from the DNA quantity data that wearer DNA could be amplified with the sample DNA of the semen. This was also verified in the M-Vac® samples for the collection of semen. While there were no full profiles that were able to be pulled out of the electropherograms for the M-Vac® samples, there were 5 partial profiles with an average number of 8 alleles being able to be pulled out of the electropherogram for the wearer of the t-shirts. As for the cutting samples, none of the samples indicated full nor partial profiles of the wearer DNA. These results for the M-Vac® and the cuttings add to the conclusion that the M-Vac® is better than the cutting method if one is trying to determine the wearer of the t-shirt as well as the semen sample on the t-shirt.

Then for the substrate control samples, 6 of the 12 samples were partial profiles with an average allele call of 9 for the wearer DNA, which means that there was at least enough DNA from the wearer of the t-shirts to be collected and amplified with both the M-Vac[®] and cutting methods and potentially affect the interpretation of the electropherograms. Ultimately though, the interpretation for these semen sample was not necessarily affected by the wearer DNA also being amplified with the semen DNA. This was likely because although there was wearer DNA being amplified, there was more DNA from the semen samples themselves.

6.2.4 Touch DNA Samples

The results from the DNA quantity data indicated that wet swabs obtained more DNA from touch DNA samples as opposed to the M-Vac[®] and were therefore more efficient. This was also not necessarily true for the touch DNA samples as well when reviewing the DNA profiles. The twelve wet swab samples for touch DNA yielded 1 full profile and 6 partial profiles with an average number of 12 alleles called, whereas the 12 M-Vac[®] samples for touch DNA did not have full profiles, but all 12 were partial profiles with an average of 20 allele calls. So, from this data, the M-Vac[®] collection method was better than the wet swab collection method. Even though a full profile was obtained with the wet swab method and a full profile was not obtained with the M-Vac[®] method, there were more partial profile for the M-Vac[®] method with a high average number of alleles called as opposed to the wet swab method.

There was also some evidence from the DNA quantity data that wearer DNA could be amplified with the sample DNA of the touch DNA. This was verified in the M-Vac[®] samples for the touch DNA since full profiles from the wearer of a t-shirt were able to be pulled out of all 12 of the touch DNA samples. As for the cutting samples, of the 12 touch DNA samples, 10 partial profiles with an average number of allele calls of 17 for the wearer DNA were able to be pulled out of the electropherograms. These results for the M-Vac[®] and the cuttings add to the conclusion that the M-Vac[®] is better than the cutting method, especially if one is trying to determine the wearer of the t-shirt as well as the touch DNA sample on the t-shirt.

Finally, for the substrate control samples, 5 of the 12 samples were full profiles for the wearers of the t-shirts and 5 of the 12 were partial profiles with an average allele call of 27 for the wearer DNA. This means that there was at least enough DNA from participants who wore the t-shirts to be collected and amplified with both the M-Vac[®] and cutting methods and potentially affect the interpretation of the electropherograms. In fact, with these samples, the interpretation for these touch DNA samples were affected by the wearer DNA also being amplified with the touch DNA. There was a lot of allelic overlap and the peaks for the allele calls for the wearer DNA were typically higher than those that were deemed to be been associated with the touch DNA sample. This was likely because there was more DNA from the wearer on the t-shirts as opposed to the touch DNA from the participant who grab the t-shirt for 30 seconds forcefully.

6.2.5 Explanation of Discrepancies and Future Directions

Some of the electropherograms had allele calls from both known and unknown sources that were not from the sample itself nor from the wearer DNA present. As previously mentioned, the t-shirts were thoroughly washed with hot water and laundry detergent. Based on the preliminary study conducted before this study, this should have left the t-shirts sterile and ready to wear with no background DNA on them. Some of the contamination can be attributed from other samples within the study or those that processed the samples, especially the substrate control sample of the t-shirts where semen was dispensed on them. As for the source of the

unknown allele calls, they could have come from people that a participant interacted with during the 12 hours that they wore a t-shirt, such as people they lived with. Participants were not told to isolate or minimize their contact with other people, but just told to go about their day as they normally would while wearing the t-shirt.

Some of the electropherograms obtained were uninterpretable. This could have occurred for a few different reasons. First, the quantity of DNA could have been too low for that sample when amplified to obtain any allele calls for a sample. Second, the sample could not be reamplified since the entire sample was consumed. This is the opposite of what was stated earlier with the cuttings and concentrations filters not being consumed entirely. The entire sample was used when necessary for amplification because the quantity values were so low for these samples that after samples were concentrated, the entire sample was amplified to determine the impact of wearer DNA on the samples. This included some substrate control samples from blood, saliva and semen, and most of the touch DNA samples for the samples collected with the M-Vac[®], with a wet swab, and the substrate controls.

In the future, a more efficient way to conduct a study like this would be to have female participant contribute a blood, saliva and touch DNA and have male participants wear a t-shirt for these sample types. Then have male participants contribute semen and have female participants wear t-shirts for this sample type. This would make it easier to determine if any wearer DNA was collected with a sample for the M-Vac[®] or a traditional method. As stated previously, with the touch DNA samples, it was a little hard to interpret samples since a lot of the alleles overlap between the touch DNA and wearer DNA, most the participants who wore t-shirts were female, and the participant who touched the t-shirts was female. Additionally, I would advise for those that are processing samples to not contribute any biological fluids, touch DNA, or wear t-shirts so that any extra allele calls from those processing samples can easily be eliminated and or accounted for from DNA profiles.

6.3 Impacts of this study

Initially, just based on the DNA quantity data, the M-Vac[®] was deemed to be less efficient than the traditional method of cuttings for the collection of blood, saliva, and semen, but more efficient for the collection of touch DNA. However, when reviewing the electropherograms, the M-Vac[®] outperformed the traditional method of cuttings for the collection of blood, saliva, and semen, and the traditional method wet swab for the collection of touch DNA. However, this does come with one drawback, as the amount of wearer DNA starts to increase in quantity to either match the quantity of an evidence sample or exceed the quantity of an evidence sample, a mixture profile is the results and it becomes harder to reliably determine who the contributor is for the sample and the person who wore the t-shirt, especially if both sources are unknown. In this case, it would be wise to use a deconvolution software to more reliably determine the major and minor contributors of a sample and the likelihood of the allele calls for each contributor, especially if it is touch DNA that is collected with the M-Vac[®] wet vacuum system.

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8. Appendices

8.1 R code for DNA Quantity Analysis

8.1.1 R code for Blood, Saliva, and Semen (All Targets)

```
#Reading in data to R
my_data <- read.csv(file.choose())
my_data

#Checking the group order
levels(my_data$Group)

# Reordering the Groups
my_data$Group <- ordered(my_data$Group,
                        levels = c("M_Vac", "Cutting", "Substrate_Control"))

#Obtaining the mean and sd of the Groups
group_by(my_data, Group) %>%
  summarise(
    count = n(),
    mean = mean(Quantity, na.rm = TRUE),
    sd = sd(Quantity, na.rm = TRUE)
  )

#Printing the box plot with points for each value
ggplot(my_data, aes(x = Group, y = Quantity, color = Group))+
  geom_boxplot()+
  geom_point(color="black", size=1, alpha=1)+
  theme(legend.position="none")+
  ylab ("Quantity (ng/uL)")
```

```

#Calculating ANOVA
res.aov <- aov(Quantity ~ Group, data = my_data)
summary(res.aov)

#Checking Homogeneity of Variance Assumption
#Printing Residue Plot
plot(res.aov, 1)

#Running Brown-Forsythe Test
leveneTest(Quantity ~ Group, data = my_data)

#Running Welch's ANOVA
oneway.test(Quantity ~ Group, data = my_data)

#Running Pairwise t-tests with the Benjamini-Hochberg adjustment
pairwise.t.test(my_data$Quantity, my_data$Group,
                p.adjust.method = "BH", pool.sd = FALSE)

#Checking Normality Assumption
#Printing Q-Q Plot
plot(res.aov, 2)

# Running Shapiro-Wilk test
aov_residuals <- residuals(object = res.aov )
shapiro.test(x = aov_residuals )

8.1.2 R code for Touch DNA (Large and Small Target)
#Reading in data to R
my_data <- read.csv(file.choose())
my_data

#Checking the group order
levels(my_data$Group)

# Reordering the Groups
my_data$Group <- ordered(my_data$Group,
                        levels = c("M_Vac", "Swabbing", "Substrate_Control"))

#Obtaining the mean and sd of the Groups
group_by(my_data, Group) %>%
  summarise(
    count = n(),
    mean = mean(Quantity, na.rm = TRUE),
    sd = sd(Quantity, na.rm = TRUE)
  )

```

```

#Printing the box plot with points for each value
ggplot(my_data, aes(x = Group, y = Quantity, color = Group))+
  geom_boxplot()+
  geom_point(color="black", size=1, alpha=1)+
  theme(legend.position="none")+
  ylab ("Quantity (ng/uL)")

#Calculating ANOVA
res.aov <- aov(Quantity ~ Group, data = my_data)
summary(res.aov)

#Checking Homogeneity of Variance Assumption
#Printing Residue Plot
plot(res.aov, 1)

#Running Brown-Forsythe Test
leveneTest(Quantity ~ Group, data = my_data)

#Running Welch's ANOVA
oneway.test(Quantity ~ Group, data = my_data)

#Running Pairwise t-tests with the Benjamini-Hochberg adjustment
pairwise.t.test(my_data$Quantity, my_data$Group,
  p.adjust.method = "BH", pool.sd = FALSE)

#Checking Normality Assumption
#Printing Q-Q Plot
plot(res.aov, 2)

# Running Shapiro-Wilk test
aov_residuals <- residuals(object = res.aov )
shapiro.test(x = aov_residuals )

8.1.3 R code for Touch DNA (Y Target)
#Reading in data to R
my_data <- read.csv(file.choose())
my_data

#Checking the group order
levels(my_data$Group)

# Reordering the Groups
my_data$Group <- ordered(my_data$Group,
  levels = c("M_Vac", "Swabbing", "Substrate_Control"))

```

```

#Obtaining the mean and sd of the Groups
group_by(my_data, Group) %>%
  summarise(
    count = n(),
    mean = mean(Quantity, na.rm = TRUE),
    sd = sd(Quantity, na.rm = TRUE)
  )

#Printing the box plot with points for each value
ggplot(my_data, aes(x = Group, y = Quantity, color = Group))+
  geom_boxplot()+
  geom_point(color="black", size=1, alpha=1)+
  theme(legend.position="none")+
  ylab ("Quantity (ng/uL)")

#Calculating ANOVA
res.aov <- aov(Quantity ~ Group, data = my_data)
summary(res.aov)

#Checking Homogeneity of Variance Assumption
#Printing Residue Plot
plot(res.aov, 1)

#Running Brown-Forsythe Test
leveneTest(Quantity ~ Group, data = my_data)

#Running Tukey HSD/Pairwise Post Hoc test
TukeyHSD(res.aov)

#Checking Normality Assumption
#Printing Q-Q Plot
plot(res.aov, 2)

# Running Shapiro-Wilk test
aov_residuals <- residuals(object = res.aov )
shapiro.test(x = aov_residuals )

```

8.2 Residual Vs. Fitted and Normal Q-Q Plots

8.2.1 Blood Samples

Figure 18. Blood Large Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 19. Blood Small Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 20. Blood Y Chromosome Target Normal Q-Q and Residual Vs. Fitted Plots

8.2.2 Saliva Samples

Figure 21. Saliva Large Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 22. Saliva Small Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 23. Saliva Y Chromosome Target Normal Q-Q and Residual Vs. Fitted Plots

8.2.3 Semen Samples

Figure 24. Semen Large Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 25. Semen Small Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 26. Semen Y Chromosome Target Normal Q-Q and Residual Vs. Fitted Plots

8.2.4 Touch DNA Samples

Figure 27. Touch DNA Large Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 28. Touch DNA Small Autosomal Target Normal Q-Q and Residual Vs. Fitted Plots

Figure 29. Touch DNA Y Chromosome Target Normal Q-Q and Residual Vs. Fitted Plots