UNIVERSITY OF CENTRAL OKLAHOMA

GRADUATE COLLEGE

EVALUATION OF THE M-VAC® SYSTEM FOR DNA COLLECTION FROM BLEACH-TREATED BLOOD STAINS: ANALYZING THE IMPACT OF BLUESTAR, MEMBRANE TYPE, AND FILTRATION

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

MASTER OF FORENSIC SCIENCE

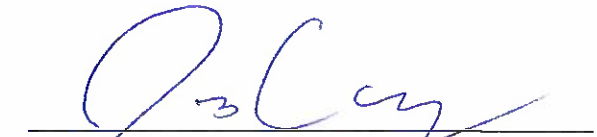
By

TERYN MARTIN
Edmond, Oklahoma
2024

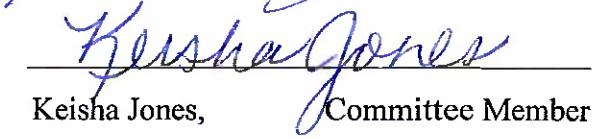
EVALUATION OF THE M-VAC® SYSTEM FOR DNA COLLECTION FROM BLEACH-TREATED BLOOD STAINS: ANALYZING THE IMPACT OF BLUESTAR, MEMBRANE TYPE, AND FILTRATION

A THESIS APPROVED FOR THE
FORENSIC SCIENCE INSTITUTE

BY



Dr. James Creecy, Committee Chairperson



Keisha Jones, Committee Member

Dr. Rhonda Williams, Committee Member

Dr. Celeste Wirsig, Committee Member

Abstract

The purpose of this experiment is to evaluate the performance of the M-Vac wet vacuum system for deoxyribonucleic acid (DNA) collection from blood-stained substrates that have been treated with bleach, a common cleaning reagent used in forensic investigations. Five variables were explored: blood concentration, bleach concentration, the presence or absence of BlueStar forensic latent bloodstain reagent, the type of membrane filter used – polyethersulfone (PES) and cellulose nitrate (CN), and the filtration product used for DNA extraction and isolation – the filter membrane itself and the filtrate. Carpet substrates were stained with single donor, whole human blood prior to treatment with bleach, which simulated cleaning the bloodstain. Appropriate samples were treated with BlueStar forensic reagent then all samples were collected with the M-Vac. The collected materials were passed over a filter with either PES or CN membrane. DNA from both the filter membranes and the filtrate were extracted, isolated, and quantified. Samples were performed in triplicate and the results, in terms of DNA quantity, underwent statistical analysis. Results indicated that the M-Vac system was capable of collecting quantifiable DNA evidence from bloodstains even after treatment with bleach. Key variances in DNA concentration between PES and CN membranes, presence and absence of BlueStar, and filters and filtrate were identified. Results demonstrated variations in DNA yield and integrity across these parameters. DNA was recovered in samples from each category of blood and bleach dilution, despite the presence of bleach. In general, BlueStar absent, CN membrane, and filter variables were found to contain higher quantities of DNA overall than their respective counterparts – BlueStar present, PES membrane, and filtrate. However, it was found that BlueStar reagent and the membrane types may have interacted with bleach or DNA extraction and quantification reagents that interfere with DNA yield. Further research is required to

determine the effect these interactions may have on forensic DNA analysis with regard to quantity and quality for compromised or degraded biological samples.

Acknowledgements

I would like to thank each committee member for their advice, encouragement, and direction throughout the course of this project. Keisha Jones played an integral part in not only the creation and execution of this thesis project, but in my acceptance into this graduate program and she has been essential to my continued progress and success. Dr. James Creecy and Dr. Rhonda Williams are remarkable educators and advisors, instilling my love for forensic DNA analysis and fostering my desire to pursue a career in the field. Dr. Celeste Wirsig has been a valued friend and mentor since before I began this graduate program, and she can be credited with inspiring me to pursue a higher education at this institution. I would also like to thank my friends and family members, especially my husband John Henry, for their endless love and support, without which none of my accomplishments would be possible. Finally, I would like to thank all other staff and faculty members of the Forensic Science Institute at the University of Central Oklahoma for supporting my personal and professional growth.

Table of Contents

Abstract ii
1. Introduction 1
1.1 The M-Vac® System 2
1.2 Forensic DNA Processing and Analysis 3
1.2.1 DNA Extraction 3
1.2.2 DNA Quantification 4
1.2.3 STR Typing 7
1.3 Hypotheses and Expected Outcomes 8
1.3.1 Effects of Bleach on DNA 8
1.3.2 Effects of BlueStar® Forensic on DNA 10
1.3.3 M-Vac® Filters and Filtrate 11
2. Methods 13
2.1 Substrate and Reagent Preparation 13
2.2 Sample Preparation 14
2.3 M-Vac Sample Collection 15
2.4 Filtrate Concentration 16
2.5 Controls 17
2.6 M-Vac Tubing Sample Collection 17
2.7 DNA Extraction and Isolation 18
2.8 DNA Quantitation 20
3. Results 22
3.1 DNA Concentration 22
3.2 Variable Comparisons 23
3.2 Percent Recovery of Experimental DNA Compared to Controls 25
3.3 Analysis of filter samples 29
3.3.1 Analysis of control filter samples 29
3.3.2 Analysis of filter samples with 1:99 bleach 30
3.3.3 Analysis of filter samples with 1:9 bleach 31
3.3.4 Analysis of filter samples with neat bleach 32
3.4 Analysis of filtrate samples 33
3.4.1 Analysis of control filtrate samples 33
3.4.2 Analysis of experimental filtrate samples 34

4. Discussion 35
4.1 Summary of Findings 35
4.2 Effects of Bleach 37
4.3 Effects of BlueStar 38
4.4 Effect of Membrane Type 39
4.5 M-Vac Tubing Study 40
4.6 Methodological Limitations 41
5. Conclusion 44
References 47
Appendix A: DNA Concentrations 53

List of Tables

Table 1. Dilution Table for Prepared Bleach and Blood 13

Table 2. Sample Groups 14

Table 3. Standard Dilution Series 20

Table 4. Average DNA Concentrations (ng/µL) 23

Table 5. Percent recovery of experimental samples compared to M-Vac control 26

Table 6. Percent recovery of all samples compared to liquid blood control 27

List of Figures

Figure 1. M-Vac Diagram 3

Figure 2. PowerPlex Fusion System STR Loci (Promega, n.d.) 8

Figure 3. Combined average DNA concentration of all groups with a single variable compared to the respective control 24

Figure 4. Combined percent recovery of experimental samples compared to M-Vac control 27

Figure 5. Combined percent recovery of experimental samples compared to liquid blood controls 28

Figure 6. Combined percent recovery of M-Vac controls compared to liquid blood controls 28

Figure 7. DNA concentration from filters of M-Vac controls 30

Figure 8. DNA concentration from filters of 1:99 bleach treated samples 31

Figure 9. DNA concentration from filters of 1:9 bleach treated samples 32

Figure 10. DNA concentration from filters of neat bleach treated samples 33

Figure 11. DNA concentration from filtrates of M-Vac controls 34

Figure 12. DNA concentration from experimental filtrates 34

1. Introduction

Biological evidence is one of many types of evidence commonly found at crime scenes and is crucial to a criminal investigation because of its potential to provide deoxyribonucleic acid (DNA) from unknown persons involved in crimes for the purpose of human identification. From bloodstains to saliva traces, DNA can be extracted from many biological sources given proper collection and processing techniques. However, advancements in DNA analysis methods and an improved understanding of the biological material by the public may drive perpetrators to tamper with or eradicate DNA evidence from crime scenes. This poses a significant challenge for investigators who endeavor to locate and collect DNA profiles of evidentiary value.

The efficacy of DNA recovery depends upon the techniques employed by crime scene investigators in collecting biological evidence. Conventional methods, including cotton swabs, adhesive tape, and scrapings, have long been utilized for DNA collection (Li & Harris, 2003; Stouder et al. 2001; Mulligan et al. 2011; Sweet et al. 2001). Yet, an emerging alternative, the Microbial Vacuum (M-Vac®) Wet-Vacuum System, presents the potential for enhanced efficiency of DNA recovery. The M-Vac® system operates as a sterile wet-vacuum apparatus, where a phosphate buffer is sprayed onto the substrate and simultaneously vacuumed into a collection bottle. The biological material is then concentrated from the collected buffer by passing through a membrane filter. The membrane filter is processed for DNA extraction in a laboratory setting, akin to conventional DNA collection methods.

While the M-Vac® system holds potential, its efficacy when used to collect biological material in the presence of bleach-based cleaning reagents and latent bloodstain visualization reagents, which may be used at a crime scene by perpetrators and investigators, respectively, remains untested. To address this gap, this project utilizes varying concentrations of human

blood and bleach on carpet substrates, treated with BlueStar® Forensic latent bloodstain reagent. The subsequent collection of biological material with the M-Vac®, followed by DNA extraction and quantification via real-time quantitative polymerase chain reaction (qPCR), will shed light on the system's performance. Comparison across variables, including blood and bleach dilutions, the presence and absence of BlueStar®, polyethersulfone (PES) and cellulose nitrate (CN) membrane types, and DNA on the membrane filter and remaining within the filtrate, will display the system's ability to yield viable DNA profiles under these conditions.

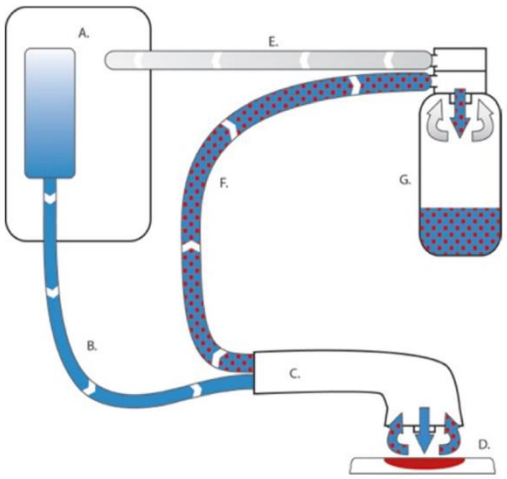
1.1 The M-Vac® System

The M-Vac® system stands as an innovative wet-vacuum tool for biological material collection. Initially developed for pathogen testing in the food industry, it has been repurposed for forensic settings, offering a unique approach to sample collection. Understanding its mechanics is paramount to preventing contamination and ensuring the quality of the collected material. The system consists of two primary components: the M-Vac® system itself and the M-Vac® apparatus. The former includes the Support Equipment Case (SEC), housing the vacuum pump and solution delivery mechanism. The latter consists of extension tubing, a collection head device, and a sample collection bottle. Additionally, the M-Vac® requires consumable products like Butterfield’s buffer and vacuum filters.

Butterfield’s buffer, a phosphate-based solution, plays a central role. Stored in a bag specifically designed for the M-Vac® system, the solution is propelled through the extension tubing by pressure from an inflated balloon. The solution is then forcefully sprayed onto the substrate via the collection head. Simultaneously, the SEC vacuums the solution containing biological material off the substrate, directing it into the collection bottle. Subsequently, the collected solution passes through a filter apparatus, which concentrates the biological material

for downstream DNA analysis procedures (M-Vac Systems®, Inc., n.d.). The filtrate which has been passed through the filter membrane would ordinarily be discarded at this stage in the process. For this project, however, the filtrate will be saved and used for DNA extraction as well. A schematic of the M-Vac® System is shown below (Figure 1).

Figure 1. M-Vac Diagram



Note. A bag of Butterfield's buffer is housed in the support equipment case (A) and is pressurized to deliver buffer to the collection head (C) through the solution line tubing (B) and is deposited onto the substrate (D). The support equipment case uses the vacuum line (E) to suck buffer from the substrate back into the collection head and through a second solution line (F). The sample is deposited into a collection bottle (G). Consumable filter is not shown. (Garrett, et al. 2014)

1.2 Forensic DNA Processing and Analysis

1.2.1 DNA Extraction

In examining DNA, the presence of cellular proteins, lipids, and other materials can obstruct accurate quantification and analysis, necessitating the isolation and extraction of DNA from the sample beforehand (Butler, 2012a). This experiment will employ the DNA IQ™ System (Promega, Madison, WI), a manual solid-phase extraction method, to extract DNA from M-Vac® filter and filtrate samples. This system uses a lysis buffer to release DNA from the

nucleus of the cell, a magnetic resin to bind the DNA while eliminating extracellular material, and an elution buffer to release DNA from the magnetic resin and retain the molecules.

The lysis buffer contains sodium dodecyl sulfate (SDS), Proteinase K, and chaotropic salts. SDS acts as a detergent, solubilizing lipids, while Proteinase K breaks down proteins. Consequently, the cell membrane, which is composed of lipids and proteins, becomes fragile. The inclusion of chaotropic salts in the lysis buffer elevates osmotic pressure, further destabilizing the cell membrane and prompting the release of DNA molecules from within the nucleus. A silica-coated paramagnetic resin is then introduced. The liberated DNA molecules bind to the magnetic resin beads which are coated in molecules that have an affinity for nucleic acids. A magnet applied to the outside of the tube holds the magnetic resin in place, while the supernatant containing the extracellular impurities is removed and discarded. While the DNA molecules are bound to the resin, a wash buffer containing ethanol is employed to cleanse the DNA and eliminate residual impurities. Finally, the addition of elution buffer increases the pH of the solution, which breaks the bonds between the DNA molecules and the magnetic resin. The DNA molecules are stored in the elution buffer and this end-product is used for downstream DNA analysis procedures.

1.2.2 DNA Quantification

After extracting DNA from a biological sample, it needs to be quantified to determine the concentration of human-specific DNA. This step is crucial for calculating the ideal amount of DNA template for downstream analysis, particularly in PCR methods. PCR is a biological reaction that amplifies specific DNA regions, generating identical copies of the original template (Butler, 2012b). The main components that go into a PCR reaction are reaction buffer, primers, DNA template, deoxynucleotide triphosphates (dNTPs), and Taq polymerase. The role of

reaction buffer is to maintain conditions for optimal operation of the Taq polymerase enzyme. Primers are synthetic molecules strategically created with sequences that bind to the regions flanking the region of interest on the DNA strands. DNA template provides the original DNA with the gene(s) or region(s) of interest that will be multiplied via the reaction and analyzed downstream. dNTPs are single nucleotide molecules, which bind together in complement to form the DNA molecule. Taq polymerase is a DNA polymerase enzyme extracted from the thermophilic bacteria, Thermus aquaticus. The enzyme synthesizes new DNA strands by attaching the complementary dNTPs in the solution to single stranded DNA.

The PCR reaction begins with an initial denaturation, which heats the double stranded DNA template and breaks the hydrogen bonds between nucleotide base pairs, resulting in two single strands of DNA. After the initial denaturation, the reaction undergoes 20-30 cycles of a three-step temperature series. The first step in the cycle is denaturation. This step further denatures the double stranded DNA into two single strands. The second step is annealing which allows the primers to locate the DNA regions flanking the region of interest and bind to them. The sequence of the primer dictates where they bind, because they are strategically created with sequences complementary to the flanking regions. The third step is elongation, during which the Taq polymerase enzyme is activated. The enzyme locates the primers, binds itself to the DNA strand, and uses the unbound dNTP molecules in the solution to synthesize and bind a new strand of DNA complementary to the original strand. The end of the elongation step marks the conclusion of a single PCR cycle. The reaction repeats, with each cycle doubling the amount of DNA present in the solution. After the last cycle of the reaction, a final extension step occurs and lasts from 5-15 minutes. This final step ensures that partial copies of DNA are completed, allows

for the PCR product to reanneal into double stranded DNA, and gives Taq polymerase additional time to completely adenylate all double stranded PCR products.

This experiment utilizes a real-time quantitative PCR (qPCR) to quantify the DNA in each sample via the Quantifiler™ Human DNA Quantification Kit from Applied Biosystems™ (Waltham, MA). qPCR observes amplification in real time, measuring the progress of the reaction after each cycle, and employs a standard curve to gauge the quantity of amplifiable DNA in the sample. Numerous factors, such as DNA degradation, PCR inhibitors, and insufficient DNA quantity, can impede PCR amplification. Therefore, qPCR assays evaluate both DNA quantity and quality for subsequent amplification steps (Butler, 2012b).

The Quantifiler™ Human DNA Quantification Kit uses a TaqMan assay, also known as a fluorogenic 5’-nuclease assay. This method involves a probe labeled with a fluorescent dye in the qPCR reaction. The probe binds to a specific DNA sequence between forward and reverse primers. Each end of the probe features a fluorescent dye – the 5’-end holds a "reporter" dye, while the 3’-end contains a "quencher" dye. Initially, the quencher suppresses fluorescence from the reporter dye. As new DNA is synthesized, the probe is cleaved, starting with the reporter dye. Once the reporter dye is liberated, it produces fluorescent light. The fluorescence is detected by the qPCR instrument and utilized with a standard curve to quantify DNA with each qPCR cycle (Butler, 2012b).

The Quantifiler™ Human DNA Quantification Kit targets two sequences. The first is a human autosomal DNA sequence 62 bp (base pairs) in length. The TaqMan probe for the human autosomal target features FAM™ fluorescent dye. Another component of the reaction is the presence of an IPC (internal PCR control), which is a synthetic sequence of DNA not found in nature. The IPC is amplified alongside the human DNA target, and the TaqMan probe for the

IPC features VIC™ dye. The function of the IPC is to verify that all assay reagents are functioning correctly. (Applied Biosystems, 2018).

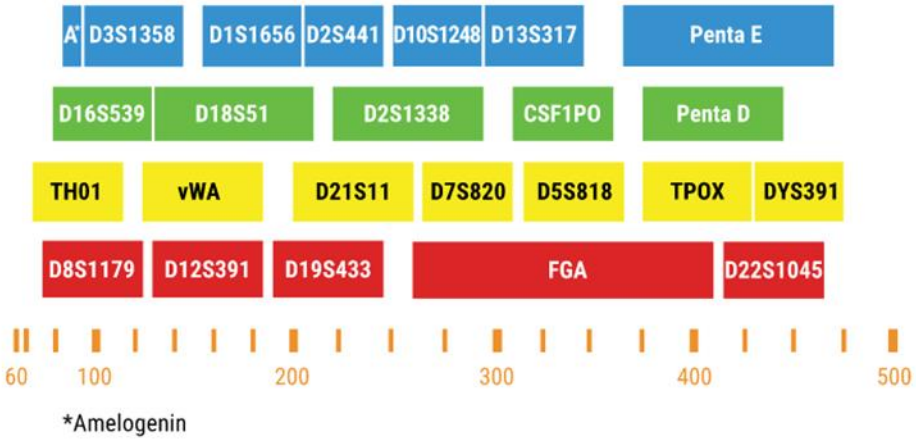
1.2.3 STR Typing

The qPCR results provide investigators with the DNA concentration in ng/µl. The concentration of DNA is crucial for determining the volume of sample to be used in subsequent PCR reactions. These reactions amplify specific DNA regions required for individual identification. While nearly all human genomes share about 99.7% similarity, certain regions contain repeated DNA sequences, valuable for forensic DNA analysis. Short tandem repeats (STRs) are among these repeated sequences, renowned for their effectiveness in individual identification due to the variability in repeat numbers among individuals (Butler, 2012c).

This experiment uses the PowerPlex® Fusion System from Promega, Madison, WI, to amplify 24 STR loci, incorporating one locus for sex determination (Promega, 2020). Many of these amplified loci possess similar sizes, necessitating the fluorescent labeling of primers to distinguish each locus. The PowerPlex® Fusion System employs five fluorescent dyes, each emitting a distinct color: Fluorescein (blue), JOE (green), TMR-ET (yellow), CXR-ET (red), and WEN (orange) (Promega, 2020).

Following amplification, the DNA fragments are size-separated via capillary electrophoresis on the ABI 3500 genetic analyzer, with labels detected using an argon laser that excites the fluorescent dye, revealing its distinct color. The figure below illustrates the 24 markers, their sizes, and their corresponding fluorescent label colors.

Figure 2. PowerPlex Fusion System STR Loci (Promega, n.d.)



1.3 Hypotheses and Expected Outcomes

1.3.1 Effects of Bleach on DNA

Bleach is a commonly used reagent in cleaning biological material and can inhibit the detection of DNA. Growing awareness of crime scene investigation techniques in terms of DNA identification has led to an increase in offenders attempting to leave as little trace of their crime as possible. Bleach has been reported to degrade DNA and as such is an ideal reagent to use when attempting to clean blood stains (Edler et al. 2020). Sodium hypochlorite (NaOCl) is the active ingredient in Clorox bleach and degrades DNA via oxidative damage, which can occur through various mechanisms. Sodium hypochlorite breaks down in water to form hypochlorous acid (HOCl) which then reacts with the DNA nucleotide bases, modifying their structures. The HOCl produced by the bleach may react further with the DNA to cleave the deoxyribose sugar backbone, leading to single-strand or double-strand breaks. This oxidative damage can further destabilize hydrogen bonding between the nucleotide base pairs leading to denaturation or fragmentation of the DNA molecule (Kemp et al. 2008).

Studies have been conducted on the effectiveness of various household cleaners on DNA in terms of crime scene evidence collection. Thabet, et al. (2018) conducted an experiment where DNA samples were collected from blood stains on cotton and silk cloth substrates that had been soaked with four different cleaning products, including Clorox bleach. Clorox bleach had the highest amount of recovered DNA from both substrates, averages of 39.17 ng/µl from cotton and 19.90 ng/µl from silk. They also found that DNA treated with Clorox was not very degraded and provided clear, crisp bands of the PCR products after gel electrophoresis. These results provided sufficient DNA for PCR amplification and indicate that investigators should still try to recover DNA from items of evidence that may have been cleansed. Similarly, Harris, et al. (2006) were able to recover DNA from blood samples treated with bleach. They applied blood samples to several substrates, allowed the sample to air dry, then cleaned the samples with chlorine bleach and other cleaning agents. They were able to extract and detect DNA from samples treated with Clorox bleach, however the DNA was progressively more degraded with increased exposure time to bleach. Ambers, et al. (2014) reported full STR profiles could be recovered from blood samples that had been immersed in 10% Clorox solution for incubation times of 1 and 2 hours.

Because sodium hypochlorite bleach will inevitably degrade much of the DNA it contacts, one way to increase DNA concentrations from blood samples treated with bleach is to simply increase the volume of biological material collected when taking a sample. The M-Vac® may provide this solution as it can collect samples from a much larger surface area and it can reach down into the pores of the substrate, where traditional DNA collection methods fall short.

All experimental samples are expected to undergo some amount of DNA degradation from bleach. Experimental samples will be compared to positive control samples that have the same blood concentration and no bleach to determine the severity of DNA degradation. The

DNA degradation may result in lower quantities of amplifiable DNA reported from qPCR. The severity of DNA degradation is expected to increase when the concentration of bleach is higher than the concentration of blood in a sample.

1.3.2 Effects of BlueStar® Forensic on DNA

BlueStar® Forensic is a presumptive test which produces a chemiluminescent reaction that indicates the presence of blood (Adams et al. 2019). It is used in this experiment because at a crime scene where blood has been cleaned with bleach, there may not be visible bloodstains. To identify possible bloodstains, the crime scene investigator would likely need to use a reagent like BlueStar® Forensic to narrow down possible areas for sampling.

BlueStar® Forensic is one of many brands of luminol-based solutions used by crime scene investigators to locate blood. It can detect blood even at miniscule dilutions of $1{:}1x10^6$. Luminol reveals blood through a chemiluminescent reaction with hemoglobin, the molecule that binds oxygen in blood, and produces a blue glow (Khan et al. 2014). A BlueStar® Forensic kit comes with luminol catalyst tablets, a bottle of chemiluminescent solution, and a trigger sprayer. The luminol catalyst tablets are dissolved in the chemiluminescent solution, then the trigger sprayer is attached to the bottle and used to spray the solution on surfaces with possible latent bloodstains.

There is little indication in the literature that luminol degrades DNA or effects DNA analysis procedures. Several experiments have been performed that confirm that luminol is safe to use in criminal investigations. Passie, et al. (2012) reported that the application of luminol to blood samples has no adverse effect on DNA analysis with PCR (Patel & Hopwood, 2013). Another study reported that full DNA profiles were able to be obtained from wet-swabs on bloodstained carpet treated with BlueStar® Forensic (Jakovich, 2012). Patel, et al. (2013),

however, reported that luminol does have an adverse effect on DNA profiling, but only with very low dilutions of blood. At a 1:990 dilution, they found significant differences between the total peak areas of control samples and luminol treated samples (Patel & Hopwood, 2013). Significant differences in the quantity and quality of DNA for samples with and without luminol of the same blood and bleach concentrations and filter types are not expected.

1.3.3 M-Vac® Filters and Filtrate

The filter that comes with the M-Vac® system has a 0.45 µm polyethersulfone (PES) membrane. PES is a common membrane type for filtering cell culture media because of its low protein binding affinity. M-Vac® Systems, Inc. also sells filter apparatuses that come with a 0.45 µm cellulose nitrate (CN) membrane. CN membranes are typically used for general laboratory filtration of buffers and solutions. Unlike the PES membrane, CN membranes have a high protein binding affinity as well as a high DNA binding affinity. This may make the CN membrane better suited than the PES membranes for filtering DNA from samples collected with the M-Vac®. The purpose of analyzing DNA collected on the filter and in the filtrate is to determine if it is advantageous to test the filtrate as well when taking samples of bloodstains that have possibly been contaminated with bleach. Bleach is known to cleave DNA into smaller fragments, therefore fragmented DNA may pass through the membrane filters and remain in the filtrate.

Prior research has been conducted to compare various membranes types in terms of DNA binding when used to filter M-Vac® samples. McLamb, et al. (2020) performed an experiment where blood samples that had been digested and undigested with an enzyme that shears DNA to produce random fragments were collected with the M-Vac® and then filtered with alternative membranes. The study compared the 0.45 µm PES membrane to 0.2 µm nylon (N), 0.2 µm CN

and 0.2 µm surfactant-free cellulose acetate (SFCA). They found that for all filter types and for both digested and undigested samples, there was significantly more DNA in the filtrate than captured on the filter membrane. They also found that for the undigested samples, the PES membrane collected the least amount of DNA, and there was a significantly increased DNA yield on the CN membrane compared to the PES membrane. However, for the digested samples, the PES membrane collected the highest amount of DNA (McLamb & Kavlick, 2020).

Based on the binding properties of the two membrane materials, the CN membrane is expected to capture higher DNA quantities than the PES membrane. M-Vac® filters are expected to yield higher DNA quantities than the filtrates in general, and specifically for non-bleach treated control samples. However, in experimental samples treated with bleach, it is plausible for the filtrates to exhibit higher DNA yields. This outcome would result from the DNA-cleaving effects of bleach, which produce smaller fragments capable of passing through the filters.

2. Methods

2.1 Substrate and Reagent Preparation

Medium-pile gray carpet substrate, obtained from Amazon, in 1-square foot tiles were cut into 3x3 inch carpet squares. The carpet squares were sterilized immediately prior to use under UV light in a Spectrolinker XL-1500 UV Crosslinker sanitizing cabinet (Spectro-UV, Farmingdale, NY) for 900 seconds at 65 J/cm^2. UV irradiation prevents contamination from DNA on the substrate prior to sampling because it causes adjacent thymine nucleotides to link together, forming a disrupted structure that prevents Taq polymerase from replicating DNA during the PCR cycle (Butler, 2012a). Dilutions of bleach (Clorox) and whole, single source, human blood (Innovative Research, Novi, MI) were prepared according to the dilution table below.

Table 1. Dilution Table for Prepared Bleach and Blood

Dilution	**Vol. Bleach/Blood**	**Vol. Water**	**Total Vol.**
Neat	6.00 mL	0.00 mL	6.00 mL
1:9	0.60 mL	5.40 mL	6.00 mL
1:99	0.06 mL	5.94 mL	6.00 mL

The blood and bleach dilutions were aliquoted into 2.0 mL microcentrifuge tubes and stored at 4° C until use. Latent bloodstain reagent was prepared immediately prior to use according to manufacturer's instructions by dissolving 3 catalyst tablets in 16 oz. lightening solution in the spray bottle provided in the BlueStar Forensic® Kit (BlueStar® Forensic, Greer, SC).

2.2 Sample Preparation

26 sample groups with variable combinations of blood concentration, bleach concentration, the presence or absence of BlueStar, and filter type were used for this experiment according to the table below:

Table 2. Sample Groups

Sample Group	Blood Concentration	Bleach Concentration	BlueStar	Filter Type
A-1	Neat	Neat	Absent	PES
A-2	Neat	Neat	Absent	CN
B-1	Neat	1:9	Absent	PES
B-2	Neat	1:9	Absent	CN
C-1	Neat	1:99	Absent	PES
C-2	Neat	1:99	Absent	CN
D-1	1:9	Neat	Absent	PES
D-2	1:9	Neat	Absent	CN
E-1	1:9	1:9	Absent	PES
E-2	1:9	1:9	Absent	CN
F-1	1:9	1:99	Absent	PES
F-2	1:9	1:99	Absent	CN
G-1	1:99	Neat	Absent	PES
G-2	1:99	Neat	Absent	CN
H-1	1:99	1:9	Absent	PES
H-2	1:99	1:9	Absent	CN
I-1	1:99	1:99	Absent	PES
I-2	1:99	1:99	Absent	CN
J-1	Neat	Neat	Present	PES
J-2	Neat	Neat	Present	CN
K-1	Neat	1:9	Present	PES
K-2	Neat	1:9	Present	CN
L-1	Neat	1:99	Present	PES
L-2	Neat	1:99	Present	CN
M-1	1:9	Neat	Present	PES
M-2	1:9	Neat	Present	CN
N-1	1:9	1:9	Present	PES
N-2	1:9	1:9	Present	CN
O-1	1:9	1:99	Present	PES
O-2	1:9	1:99	Present	CN
P-1	1:99	Neat	Present	PES
P-2	1:99	Neat	Present	CN
Q-1	1:99	1:9	Present	PES

Q-2	1:99	1:9	Present	CN
R-1	1:99	1:99	Present	PES
R-2	1:99	1:99	Present	CN
S-1	Neat	None	Present	PES
S-2	Neat	None	Present	CN
T-1	Neat	None	Absent	PES
T-2	Neat	None	Absent	CN
U-1	1:9	None	Present	PES
U-2	1:9	None	Present	CN
V-1	1:9	None	Absent	PES
V-2	1:9	None	Absent	CN
W-1	1:99	None	Present	PES
W-2	1:99	None	Present	CN
X-1	1:99	None	Absent	PES
X-2	1:99	None	Absent	CN
Y-1	None	None	Present	PES
Y-2	None	None	Present	CN
Z-1	None	None	Absent	PES
Z-2	None	None	Absent	CN

For each sample, 100 uL of the appropriate blood concentration (neat, 1:9, 1:99, or none) was applied to the center of a sterilized carpet square with a micropipette. The blood stains were allowed to air dry for 24 hours. 100 µL of the appropriate bleach concentration (neat, 1:9, 1:99, or none) was applied to the blood stain with a micropipette. Using a kim wipe, the bleach was rubbed into the blood stain in 5 circular motions with even pressure. The "cleaned" blood stains were allowed to air dry for 24 hours. For samples with BlueStar present, the carpet square was photographed under overhead lighting. Then BlueStar latent bloodstain reagent was sprayed 1x onto the center of the carpet square. The chemiluminescent reaction was photographed in darkness.

2.3 M-Vac Sample Collection

The M-Vac was used to collect samples from the entire carpet square by passing the M-Vac head over the surface of the carpet square in successive, overlapping lines with solution

pressure and vacuum on until 50 mL of M-Vac solution had accumulated in the collection bottle. The collection bottle was removed from the M-Vac and the collected sample was transferred to a 50 mL conical tube. One M-Vac head and collection bottle was used for all samples in the same letter group designation (samples in groups A-1 and A-2 were both collected using the same M-Vac head and collection bottle [Table 2]) because samples in groups with the same letter designation contained the same variables for blood concentration, bleach concentration, and BlueStar use. The sample was then vacuum filtered with the appropriate membrane type (PES or CN) by pouring the entire sample from the 50 mL conical tube into the filter apparatus and then applying vacuum suction to the apparatus. The filtrate from the samples were transferred back to a 50 mL conical tube until further use. The membranes were removed from the filter apparatus and allowed to air-dry, then stored at room temperature until further use.

2.4 Filtrate Concentration

The filtrates were concentrated using 30 kDa molecular weight cut off (MWCO) Amicon® Ultra-15 Centrifugal Filters (Millipore Sigma, Burlington, MA). 30 kDa MWCO means that the filter can retain molecules with a molecular weight larger than 30,000 Daltons (Da). A single DNA base pair has an average molecular weight of 650 Da (Alberts, 2014), therefore the 30 kDa MWCO filters could retain DNA fragments as small as 46 bp. The PES and CN M-Vac membrane filters are different from the 30 kDa MWCO filters in that the pore size (0.45 µm) is measured in diameter, not molecular weight cut off.

15 mL filtrate was placed into a centrifugal filter unit and centrifuged for 10 minutes at 4,000 x g. Filtrate from the centrifugal filter unit was discarded and remaining M-Vac sample filtrate was added to the centrifugal filter unit and centrifuged again for 10 minutes at 4,000 x g. This was repeated until all M-Vac sample filtrates were processed and the total volume of

concentrated M-Vac filtrate was below 1.5 mL. The concentrated samples were transferred to 2.0 mL microcentrifuge tubes and stored at 4° C until further use.

2.5 Controls

Two types of controls were used in this experiment, M-Vac controls and liquid blood controls. The M-Vac controls were collected with the M-Vac in the same manner as all experimental samples, but without the use of bleach. The M-Vac controls can be further broken down into positive and negative controls. The positive controls contained all variable combinations except for bleach, and the negative controls contained neither bleach nor blood. The positive M-Vac controls provide data pertaining to how the M-Vac can be expected to perform under these conditions without the complication of bleach degradation, while the negative M-Vac controls were used to ensure that the substrates were thoroughly disinfected prior to sampling and that no contamination was introduced during the collection process.

The liquid blood controls are 100 µL of the three separate concentrations of liquid blood itself undergoing DNA extraction and quantification with the same methods as the experimental samples. The liquid blood controls were not subjected to carpet substrate, or collected with the M-Vac. These controls were used to determine how much DNA is present in 100 µL of the liquid blood concentrations and would be present in the sample before the addition of bleach or BlueStar and collection with the M-Vac.

2.6 M-Vac Tubing Sample Collection

Tubing that carried samples from the M-Vac head to the M-Vac collection bottle was cut off the used M-Vac heads to allow access for swabbing the interior of the tubing. This was done in order to determine how much DNA would be left behind in the sterile tubing after sampling with M-Vac. The tubing was swabbed with one wet sterile cotton tipped swab (Arrowhead

Forensics, Lenexa, KS), then again with one dry swab. The swabs were stored in their swab sleeve at room temperature until further use.

2.7 DNA Extraction and Isolation

Because the M-Vac membranes were too large to process the entire membrane for DNA isolation, three small punches from each membrane were taken near the center of each membrane using a standard 6-mm single-hole punch. Because of the diameter of the PES membranes (50 mm) are different than the CN membranes (45 mm), three 6-mm hole punches accounts for 4.32% of the whole PES membrane and 5.33% of the whole CN membrane. This membrane size discrepancy is discussed further in section 4.6 Methodological Limitations.

The hole punch was cleaned by immersion in 10% bleach followed by 100% ethanol between samples to limit cross contamination. The three membrane punches were placed into a 2.0 mL microcentrifuge tube for DNA isolation. The remaining membranes were returned to storage at room temperature. The volumes of the concentrated M-Vac filtrates were normalized by bringing the volume of each sample to 1.5 mL with Butterfield's buffer (solution used by the M-Vac to collect samples). Half of the sample volume, 750 µL, was transferred to a new 2.0 mL microcentrifuge tube for DNA isolation. The remaining concentrated filtrate was returned to storage at 4°C. Half of the cotton tips from both swabs used to sample the interior of the M-Vac tubing were cut off and placed into a 2.0 mL microcentrifuge tube for DNA isolation. The remaining cotton swabs were returned to their swab sleeves and stored at room temperature.

All samples were processed for DNA isolation using the DNA IQ™ System (Promega, Madison, WI) according to manufacturer's instructions for DNA Isolation for Stains and Buccal Swabs (Promega, 2016). A master mix of lysis buffer and DTT was prepared by combining 250 µL lysis buffer provided in the DNA IQ™ System kit with 2.5 µL 1M DTT for each sample

being processed, plus 10% additional samples to account for pipetting error (when processing 30 samples, master mix was calculated for 33 samples). 252.5 µL prepared lysis buffer was added to each sample, including one reagent blank (RB) for the batch being processed. The RB consisted of prepared lysis buffer in a clean, empty microcentrifuge tube and was processed along with all other samples in the batch. The samples were incubated at 70˚C for 30 minutes in a dry heat block (Corning, Glendale, AZ).

For the samples that contained a solid substrate (M-Vac membrane punches and tubing swabs), the substrates were removed from the prepared lysis buffer and placed into a spin basket. The spin baskets were returned to the microcentrifuge tube. The sample tubes were centrifuged at maximum speed for two minutes using a tabletop centrifuge (Eppendorf, Enfield, CT). The spin baskets containing substrates were removed and discarded.

7 µL DNA IQ™ magnetic resin was added to each sample. The sample tubes were incubated at 65˚C for 5 minutes in a dry heat block, with brief vortexing every minute. The sample tubes were then placed into a magnetic stand. With the magnetic resin pooled on the side of the tube, the prepared lysis buffer was removed and discarded. A second lysis buffer master mix was prepared by combining 100 µL lysis buffer provided in the DNA IQ™ kit with 1 µL 1M DTT for each sample, plus 10%. 101 µL prepared lysis buffer was added to each sample. The sample tubes were vortexed and returned to the magnetic stand. The prepared lysis buffer was removed and discarded.

1X wash buffer was prepared by adding 35 mL 100% ethanol and 35 mL isopropyl alcohol to the 70 mL 2X wash buffer provided in the DNA IQ™ kit. The samples were washed thrice by adding 100 µL 1X wash buffer, vortexing, then removing and discarding the wash buffer. With the sample tubes still in the magnetic stand, the tubes were opened, and the resin

was allowed to air-dry for 5 minutes. 50 µL elution buffer was added to each sample and vortexed. The sample tubes were incubated at 65˚C for 5 minutes in a dry heat block, then they were returned to the magnetic stand and the elution buffer was removed and placed into a clean 0.2 mL microcentrifuge tube. The elution containing the isolated DNA was stored at -20˚C until further use.

2.8 DNA Quantitation

The isolated DNA samples were quantified via qPCR using the Quantifiler™ Human DNA Quantification kit (Applied Biosystems™, Waltham, MA), according to manufacturer's instructions (Applied Biosystems, 2018). An eight-point standard dilution series was prepared according to Table 3 below. The DNA standards were prepared from 200 ng/µL stock DNA, which is included in the Quantifiler™ Human DNA Quantification kit. The stock DNA standard is pooled, human genomic DNA with a known concentration and is serially diluted to make a set of standards and that have a linear relationship. The standards are amplified via real-time qPCR along with unknown samples, and the concentration of the unknown samples is mathematically extrapolated by comparing the fluorescence of the unknown samples to the fluorescence of the standards.

Table 3. Standard Dilution Series

Standard #	**Concentration (ng/µL)**	**Standard volume**	**TE Buffer volume**
Std. 1	50.000	10 uL stock (200 ng/µL)	30 uL
Std. 2	16.700	10 uL Std. 1	20 uL
Std. 3	5.560	10 uL Std. 2	20 uL
Std. 4	1.850	10 uL Std. 3	20 uL
Std. 5	0.620	10 uL Std. 4	20 uL
Std. 6	0.210	10 uL Std. 5	20 uL
Std. 7	0.068	10 uL Std. 6	20 uL
Std. 8	0.023	10 uL Std. 7	20 uL

A PCR master mix was prepared by combining 10.5 µL Quantifiler™ Human Primer Mix and 12.5 µL Quantifiler™ PCR Reaction Mix for each sample, plus an additional 10% volume to account for pipetting error. 23 µL PCR master mix was dispensed into all wells of a Micro-Amp™ Optical 96-Well Reaction Plate (Applied Biosystems, Waltham, MA). 2 µL of standard or isolated DNA was added to each well. Each standard was run in duplicate per reaction plate. One well on each plate was left without any DNA template from standards or isolated DNA samples to serve as a non-template control (NTC). The plate was covered with a Micro-Amp™ Optical Adhesive Film (Applied Biosystems, Waltham, MA) and centrifuged briefly to remove bubbles from reaction wells.

The 96-well plate was placed into CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, CA) and the following thermal cycling conditions were used: 1 cycle of 95.0˚C for 10:00 minutes; 40 cycles of 95.0˚C for 1:15 minutes and 60.0˚C for 1:00 minute. All fluorophore channels were selected for detection before beginning the thermal cycling program. qPCR data was exported from the instrument and analyzed using BioRad CFX Manager Software.

3. Results

3.1 DNA Concentration

Data for all individual samples, including concentrations from M-Vac filters and filtrates, swabs from the M-Vac tubing, and liquid blood (LB) controls for each blood concentration are presented in Appendix A. Samples 32, 64, 96, 128, 161, 193, 225, 257, 289, 322, and 354 are reagent blank controls for the DNA extraction protocol and were not included in the appendix. Table 4 below shows the average DNA concentration for each group, allowing for a straightforward comparison of the variables.

Samples indicated with an asterisk (*) in the sample column of Appendix A are outliers and were not included in data analysis. Outliers in this study were identified using contextual evaluation rather than a statistical method such as the interquartile range (IQR). While the IQR method is a strong statistical tool, applying it in this context would have resulted in the exclusion of a significant portion of the true data collected in this experiment. Instead, potential outliers were assessed based on their magnitude relative to the typical experimental value. Data points that were orders of magnitude higher than the typical value were excluded from analysis. 98% of all M-Vac filter and filtrate samples had a DNA concentration value between 0 ng/µL and 0.21696 ng/µL. The five samples that were determined to be outliers had DNA concentration values between 61 ng/µL and 45,340,144 ng/µL and are not representative of the true data in this experiment. The reason behind the occurrence of these outliers was not determined, but there are a variety of technical issues that could have caused the inflated DNA quantities, including detection artifacts, such as optical interference, and errors in data analysis, such as incorrect threshold settings.

Table 4. Average DNA Concentrations (ng/µL)

	Filter				Filtrate			
	PES Membrane		CN Membrane		PES Membrane		CN Membrane	
[Blood] | [Bleach]	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**
Neat | None	0.05806	0.05282	0.11682	0.09902	0.00169	0.00135	0.00407	0.00276
Neat | 1:99	0.11415*	0.01439	0.17097*	0.10092*	0.00066	0.00833*	0.00052	0.06847*
Neat | 1:9	0.05668	0.04402	0.16042*	0.08128	0	0.00052	0.00059	0.00238
Neat | Neat	0.00400	0.00342	0.00414	0.02101	0.00303*	0.00172*	0.00272	0
1:9 | None	0.00696	0	0.00681	0.00046	0	0.00297	0.00154	0.00068
1:9 | 1:99	0.00083	0.00050*	0.00072	0	0.00139*	0	0	0.00140*
1:9 | 1:9	0.00166	0	0.00034	0	0	0	0	0.00182*
1:9 | Neat	0	0	0.00078	0	0	0.00788*	0	0
1:99 | None	0	0	0	0	0	0.00035	0.00072	0.00146
1:99 | 1:99	0.00045*	0.00102*	0	0	0.00054*	0.00035	0	0.00107
1:99 | 1:9	0.00069*	0.00047*	0.00157	0	0	0	0.00343*	0
1:99 | Neat	0	0	0	0	0.00237*	0	0.00128*	0.00033

Note. n = 3. Gray shading indicates M-Vac control groups (groups that were not treated with bleach)

* Experimental value greater than control value.

3.2 Variable Comparisons

Figure 3 presents combined average DNA concentrations of experimental and control groups for the three variable distinctions (filter vs. filtrate sample types, PES vs. CN membranes, and presence vs. absence of BlueStar reagent).

Figure 3. Combined average DNA concentration of all groups with a single variable compared to the respective control

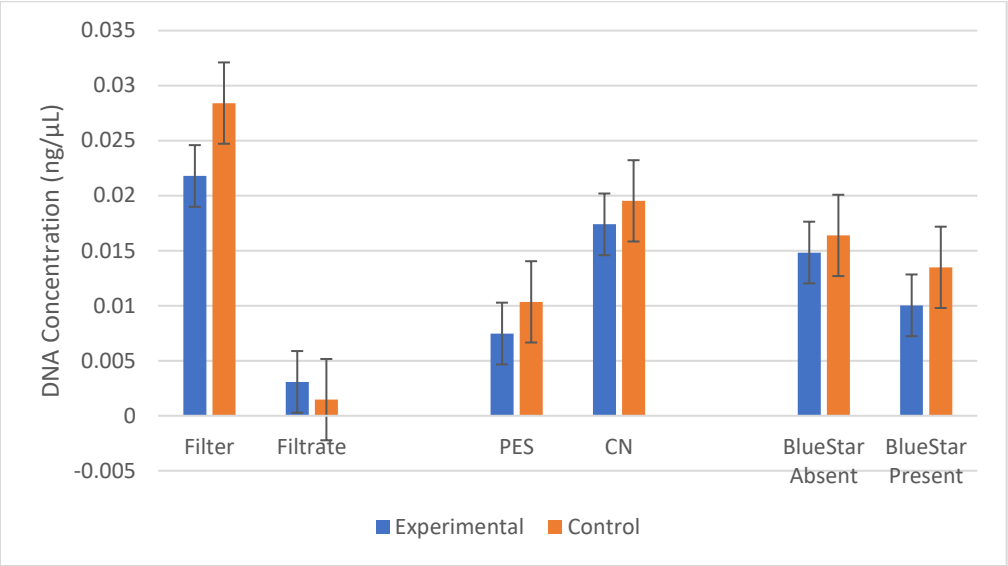


Figure 3 was created by calculating the average of all experimental group values in Table 4 with a single variable (ex. all experimental filter samples) and comparing it to the average of the M-Vac control group values of the same variable. Experimental groups are those with any concentration of bleach present. The M-Vac control groups are those with no bleach present and are indicated in Table 4 with gray shading. This figure gives a brief overview of how each variable performed compared to its counterpart variable (filter vs. filtrate/ PES vs. CN/ BlueStar Absent vs. BlueStar Present) and compared to its respective control but does not speak to the combined effects of the variables or the effects from differences in blood and bleach concentrations.

All experimental variables overall exhibited lower concentrations of DNA than its control counterpart, except the filtrate samples. Filtrate groups exhibiting higher DNA concentrations in experimental groups than control groups which was not unexpected due to the effect of bleach on DNA molecules. As bleach reacts with blood, it cleaves DNA molecules into smaller pieces that

can more easily pass through the pores of the membrane filter. Therefore, samples with bleach would be expected to have more DNA present in the filtrate than samples without bleach.

3.2 Percent Recovery of Experimental DNA Compared to Controls

The M-Vac controls, which were samples taken with the M-Vac with all variable combinations but bleach for the positive controls, and without bleach or blood for the negative controls, were used to determine how well the M-Vac performs under these conditions without bleach-induced DNA degradation. The liquid blood controls in this experiment were used to determine how much DNA should theoretically be present on the substrate prior to chemical treatment and M-Vac collection for each blood concentration. These are samples 323, 324, and 325 presented in Appendix A. The neat liquid blood control contained 0.38974 ng/µL DNA, the 1:9 liquid blood control contained 0.29635 ng/µL DNA, and the 1:99 liquid blood control contained 0.10449 ng/µL DNA. As expected, the concentration of DNA in the liquid blood decreased along with the liquid blood concentration, with neat liquid blood containing the highest concentration of DNA and 1:99 liquid blood containing the lowest concentration of DNA. Percent recovery data of experimental samples from both control types are presented here.

Table 5 shows the percent recovery of DNA from each group compared to its M-Vac control. These values were derived from Table 4. The value for a single group was divided by its respective control group value – the group with the same variable combination but without bleach present. The groups with no value present could not be calculated because the average DNA concentration for the control group was 0 ng/uL.

Table 5. Percent recovery of experimental samples compared to M-Vac control

	Filter				Filtrate			
	PES Membrane		CN Membrane		PES Membrane		CN Membrane	
[Blood] | [Bleach]	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**
Neat | 1:99	196.6%	27.2%	146.4%	101.9%	39.1%	617.0%	12.8%	2480.8%
Neat | 1:9	97.6%	83.3%	137.3%	82.1%	0.0%	38.5%	14.5%	86.2%
Neat | Neat	6.9%	6.5%	3.5%	21.2%	179.3%	127.4%	66.8%	0.0%
1:9 | 1:99	11.9%	-	10.6%	0.0%	-	0.0%	0.0%	205.9%
1:9 | 1:9	23.9%	-	5.0%	0.0%	-	0.0%	0.0%	267.6%
1:9 | Neat	0.0%	-	11.5%	0.0%	-	265.3%	0.0%	0.0%
1:99 | 1:99	-	-	-	-	-	100.0%	0.0%	73.3%
1:99 | 1:9	-	-	-	-	-	0.0%	476.4%	0.0%
1:99 | Neat	-	-	-	-	-	0.0%	177.8%	22.6%

Figure 4 was derived from Table 5 and presents the combined percent recovery of DNA for a single variable compared to its M-Vac control. It was created by averaging the values in Table 5 for a single variable (filter, filtrate, PES, etc.). This data is notable because many of the variables exhibit percent recoveries of over 100%. Many groups exhibit percent recoveries over 100% indicating that those samples collected higher concentrations of DNA than their no-bleach controls. As discussed earlier, this phenomenon was anticipated with filtrate samples due to bleach cleaving DNA into smaller pieces which could pass through the membrane filter pores more easily. However, this result was not expected in filter samples. A possible explanation for this result among filters might be from unintentional variances in the M-Vac sampling process. Section 4.6. Methodological Limitations further discusses this discrepancy in the sampling procedure along with other methodological limitations that could contribute to unexpected findings.

Figure 4. Combined percent recovery of experimental samples compared to M-Vac control

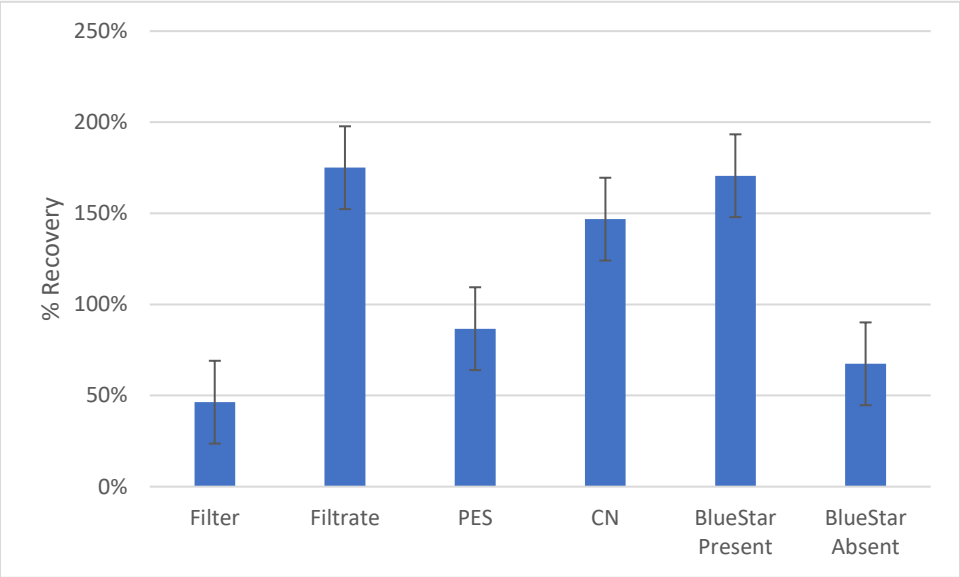


Table 6 shows the percent recovery of each group compared to its liquid blood control. The values were derived from the values in Table 4 and the liquid control values in Appendix A (samples 323, 324, and 325) by dividing the values in Table 4 by the liquid blood control of the same concentration. This table includes the percent recovery of the M-Vac controls compared to the liquid blood controls, which are shaded in gray.

Table 6. Percent recovery of all samples compared to liquid blood control

	Filter				**Filtrate**			
	PES Membrane		**CN Membrane**		**PES Membrane**		**CN Membrane**	
[Blood] | [Bleach]	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**	**BlueStar Absent**	**BlueStar Present**
Neat | None	14.9%	13.6%	30.0%	25.4%	0.4%	0.3%	1.0%	0.7%
Neat | 1:99	29.3%	3.7%	43.9%	25.9%	0.2%	2.1%	0.1%	17.6%
Neat | 1:9	14.5%	11.3%	41.2%	20.9%	0%	0.1%	0.2%	0.6%
Neat | Neat	1.0%	0.9%	1.1%	5.4%	0.8%	0.4%	0.7%	0%
1:9 | None	2.3%	0%	2.3%	0.2%	0%	1.0%	0.5%	0.2%
1:9 | 1:99	0.3%	0.2%	0.2%	0%	0.5%	0%	0%	0.5%
1:9 | 1:9	0.6%	0%	0.1%	0%	0%	0%	0%	0.6%
1:9 | Neat	0%	0%	0.3%	0%	0%	2.7%	0%	0%
1:99 | None	0%	0%	0%	0%	0%	0.3%	0.7%	1.4%
1:99 | 1:99	0.4%	1.0%	0%	0%	0.5%	0.3%	0%	1.0%
1:99 | 1:9	0.7%	0.4%	1.5%	0%	0%	0%	3.3%	0%
1:99 | Neat	0%	0%	0%	0%	2.3%	0%	1.2%	0.3%

Note. Gray shading indicates M-Vac control groups

Figure 5. Combined percent recovery of experimental samples compared to liquid blood controls

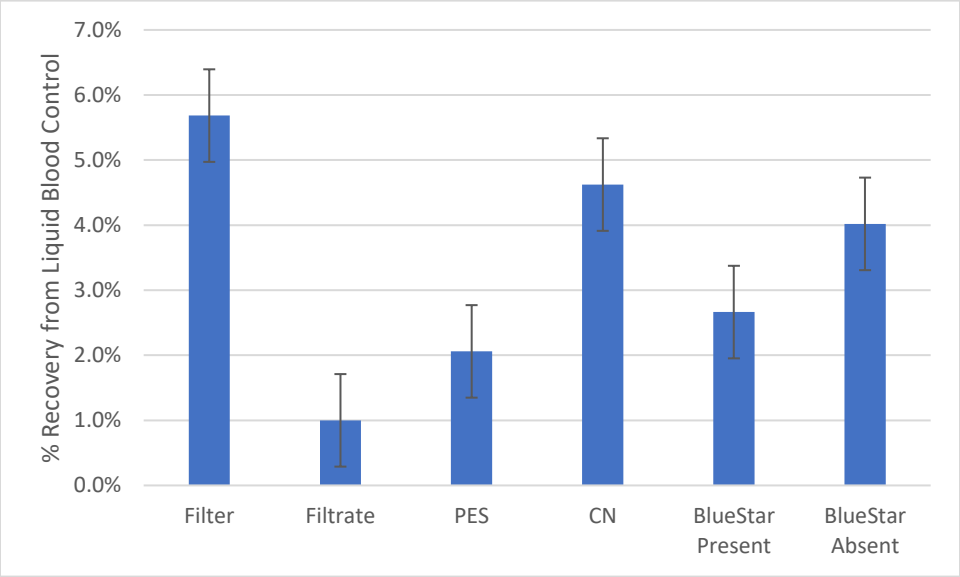
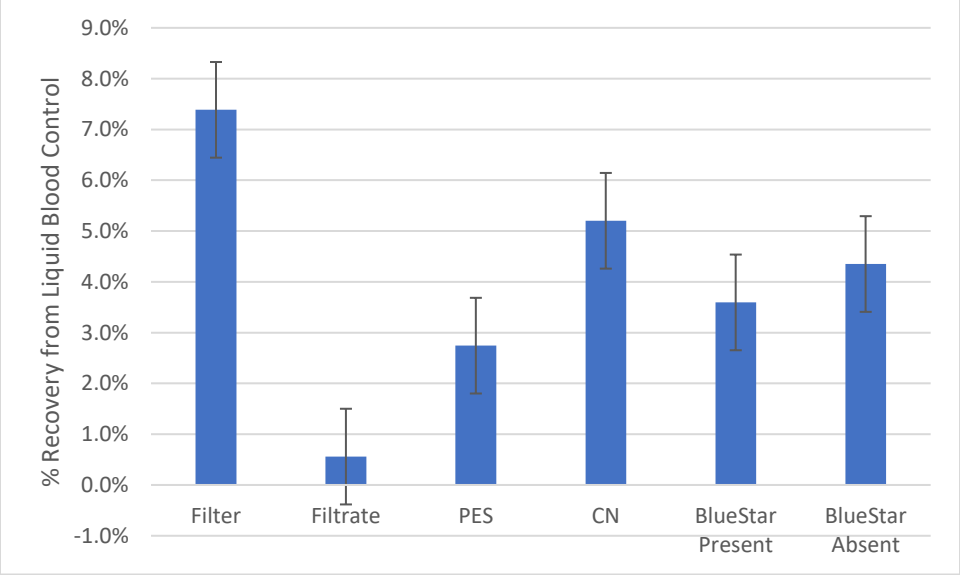


Figure 6. Combined percent recovery of M-Vac controls compared to liquid blood controls



Figures 5 and 6 show the combined percent recovery of the experimental samples compared to the liquid blood controls and the percent recovery of the M-Vac controls from liquid blood controls, respectively. Liquid blood controls were included in this study in addition to the M-Vac controls to gauge how much of the DNA from 100 µL of each blood dilution (neat,

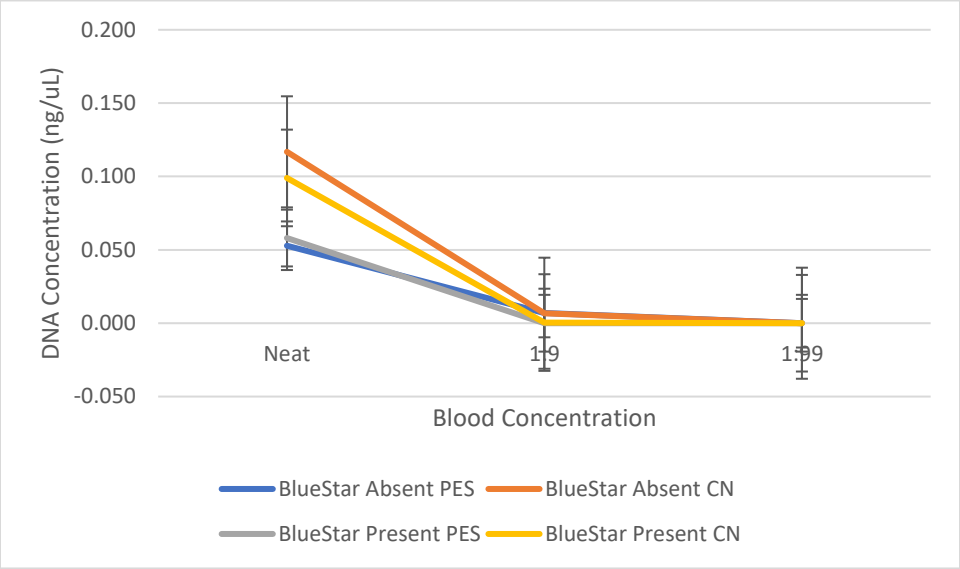
1:9, and 1:99) was present on the substrate and could have been collected by the M-Vac, regardless of other variables applied. The trends in percent recovery data from liquid blood are similar between experimental samples and M-Vac controls – with the filter, CN, and BlueStar absent samples yielding higher percent recoveries than filtrate, PES, and BlueStar present samples.

3.3 Analysis of filter samples

3.3.1 Analysis of control filter samples

Figure 7 shows the variances in amount of DNA collected from the filters of the M-Vac control groups. Each line on the graph represents a different combination of the BlueStar and filter type variables. The x-axis represents DNA concentration in ng/µL and each point on the y-axis represents the blood concentration applied to the substrate. As expected, the DNA concentrations for all variable combination groups decreased with the concentration of blood. The neat blood sample groups with CN membrane notably have higher DNA concentrations than the groups with PES membrane, though there is not a significant difference between the membrane types among the 1:9 and 1:99 blood concentration groups.

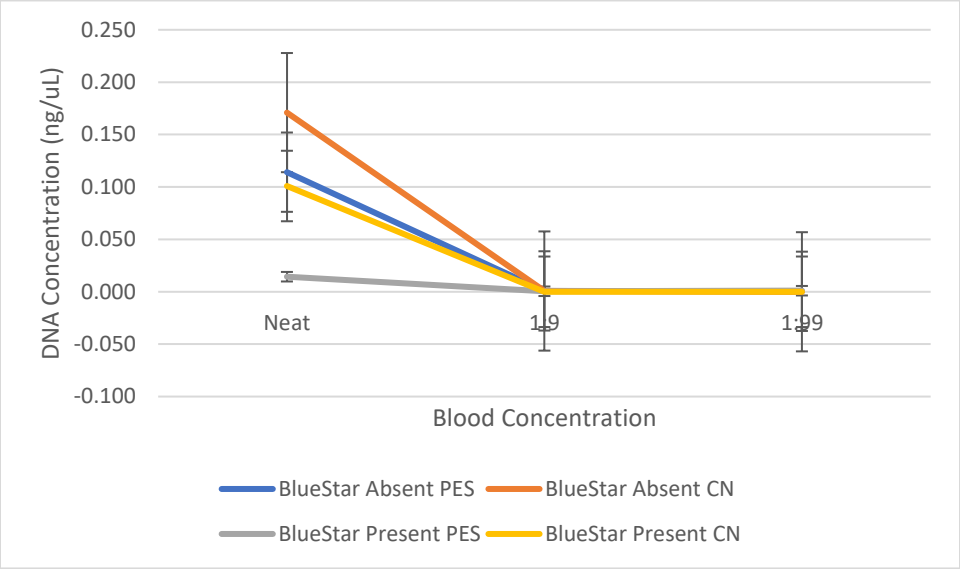
Figure 7. DNA concentration from filters of M-Vac controls



3.3.2 Analysis of filter samples with 1:99 bleach

Figure 8 displays DNA concentrations from M-Vac samples treated with 1:99 bleach. These groups were expected to have results similar to the control groups shown in Figure 7 because these groups contain the lowest bleach concentration. Like the controls, DNA concentration in these groups decreases with blood concentration. Among the samples with neat blood in Figure 8, the group with BlueStar absent and CN membranes shows the highest concentration of DNA, which is also true for the neat blood controls in Figure 7. However, a notable difference between Figures 7 and 8 is that in Figure 8, the neat blood samples with CN membranes do not have higher DNA concentrations than the samples with PES membranes for both BlueStar variables. Instead, Figure 8 shows that both groups with BlueStar absent have higher DNA concentration than the groups with BlueStar present. This indicates that there may be an interaction between BlueStar reagent and bleach that affects DNA concentration.

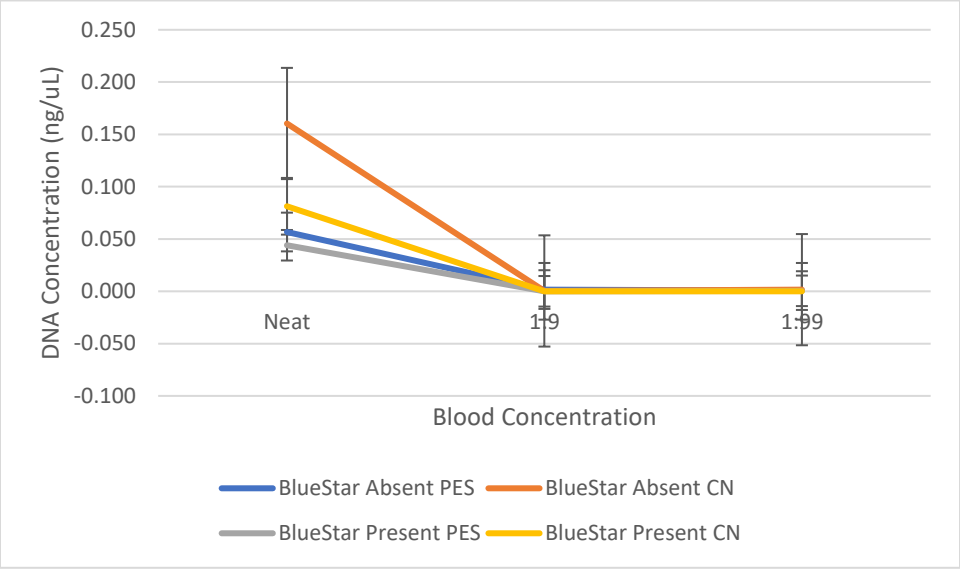
Figure 8. DNA concentration from filters of 1:99 bleach treated samples



3.3.3 Analysis of filter samples with 1:9 bleach

Figure 9 contains DNA concentrations from M-Vac samples treated with 1:9 bleach. Like the M-Vac control filters (Figure 7), the DNA concentrations in Figure 9 decrease with decreasing blood concentration. Comparing Figure 9 to Figure 7 also demonstrates that for both controls and 1:9 bleach, the groups with CN membranes yield higher DNA concentrations than the groups with PES membranes within the neat blood concentration groups. Another notable feature of Figure 9 is that the groups with BlueStar absent have higher DNA concentrations than the groups with BlueStar present, regardless of the filter membrane type used. This result supports the findings from Figure 7 that CN membranes collect more DNA than PES membranes, and from Figure 8 that samples with BlueStar absent have more DNA than samples with BlueStar present.

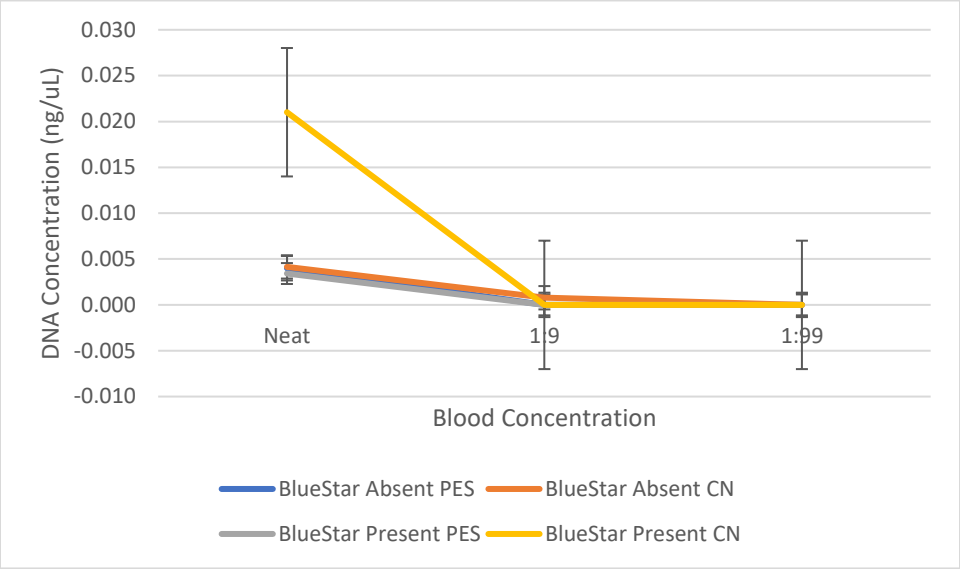
Figure 9. DNA concentration from filters of 1:9 bleach treated samples



3.3.4 Analysis of filter samples with neat bleach

DNA concentration from the filters of sample groups treated with neat bleach are shown in Figure 10. These sample groups were expected to have low DNA yields, regardless of the blood concentration, due to having the highest concentration of bleach. All filter samples have low DNA concentrations that are equal to or near 0 ng/µL, except for the group with BlueStar present and CN membrane at the neat blood concentration. This group yielded 0.021 ng/µL DNA, which is 5.25x greater than the next highest DNA concentration among these groups. There are no other apparent trends regarding BlueStar presence or filter membrane type in Figure 10. This is likely due to the high concentration of bleach present in these samples, which demonstrates that high concentrations of bleach can significantly disrupt DNA yield, regardless of the filter membrane type or the presence of BlueStar utilized in the collection process.

Figure 10. DNA concentration from filters of neat bleach treated samples



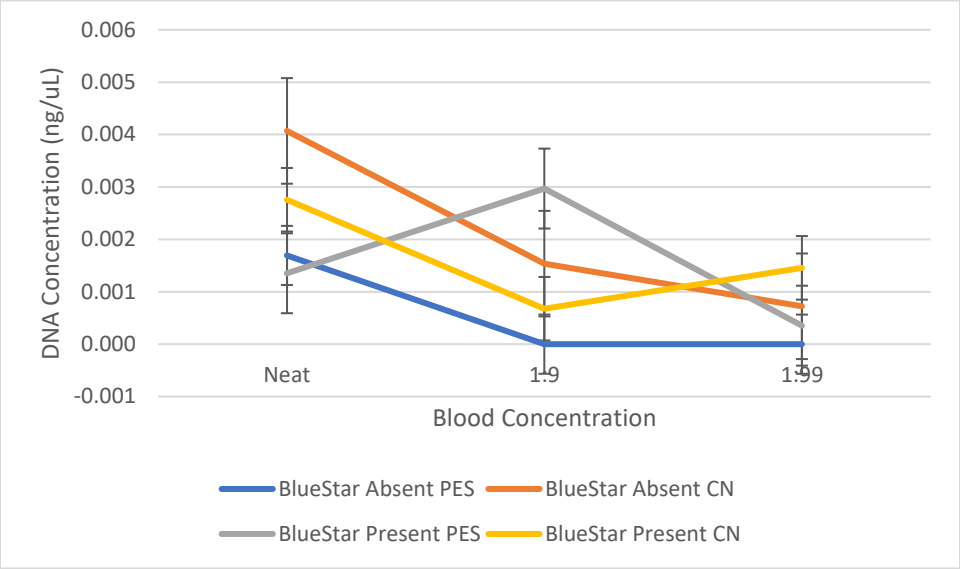
Note. The BlueStar Absent PES line (blue) is located behind BlueStar Present PES (gray) and BlueStar Absent CN (orange) lines.

3.4 Analysis of filtrate samples

3.4.1 Analysis of control filtrate samples

Figure 11 shows the DNA concentration from filtrates of samples from the bleach-less control groups. DNA concentration for these samples were also expected to decrease with decreasing blood concentration. The groups with BlueStar absent follow the trend, but the groups with BlueStar present do not. This may indicate that the presence of BlueStar influences DNA concentration from M-Vac filtrate.

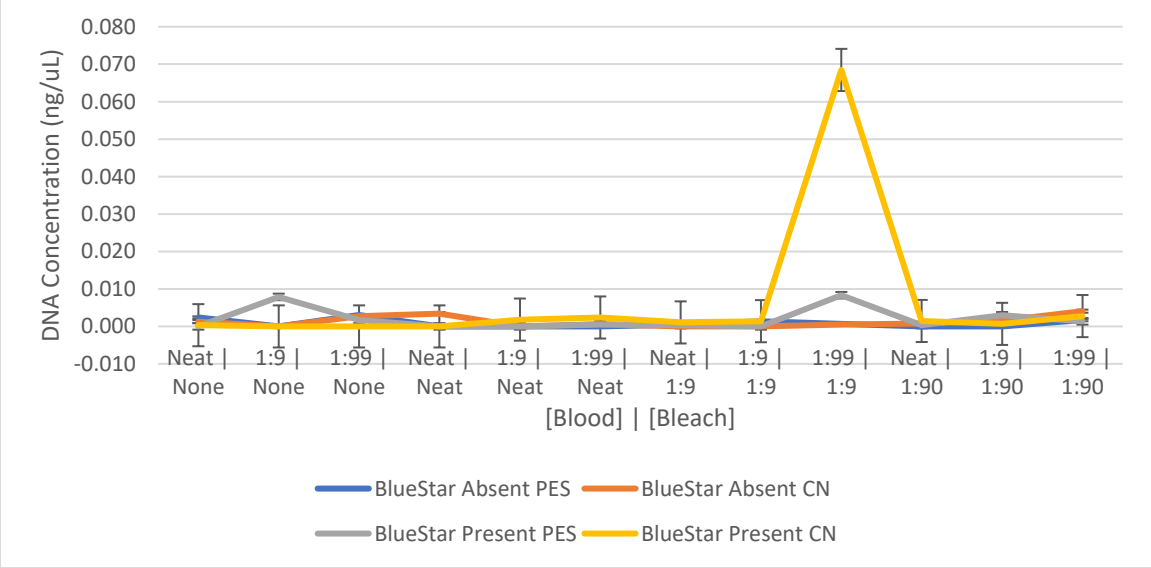
Figure 11. DNA concentration from filtrates of M-Vac controls



3.4.2 Analysis of experimental filtrate samples

Figure 12 shows the DNA concentration of all experimental filtrate groups. The x-axis represents DNA concentration, and the y-axis represents each group by concentration of blood and bleach present, with the blood concentration listed above the bleach concentration. The experimental filtrate groups showed no consistent results or trends and were not analyzed further.

Figure 12. DNA concentration from experimental filtrates



4. Discussion

4.1 Summary of Findings

The results show that all variables present in this experiment have a distinct influence on the amount of DNA recovered. Blood concentration present in the sample initially directly impacts the amount of DNA recovered because it dictates how much DNA exists on the sample prior to the use of any chemical reagents and DNA collection methods. The results showed that DNA concentration decreased as the blood concentration decreased, regardless of all other variables.

The impact of bleach concentration can be seen in the differences between control groups and experimental groups among filters and filtrates. Among the filter groups, DNA concentration in the controls groups were generally higher than experimental groups with all other variables the same, with some exceptions. 9 out of the 36 experimental filter groups had higher DNA concentrations than the respective control group. These groups are indicated with an asterisk in Table 4. It was not expected for any experimental group to have a higher DNA yield than its control group, however these results could be due to inconsistencies in the M-Vac collection process. Although even pressure was applied while rubbing bleach into the bloodstained substrate, the same amount of pressure may not have been used for every sample. Pressure variations when "cleaning" the substrate could account for the unexpected results.

BlueStar latent blood reagent may have an impact on DNA yield as well. BlueStar states that the reagent does not interfere with DNA typing, and while this study did not analyze the quality of DNA typing results and therefore cannot confirm or deny the claim, it is evident that the presence of BlueStar may have an impact on the quantity of DNA collected. As seen in Figure 3, both experimental and control samples with BlueStar absent have higher DNA yields

than samples with BlueStar present. When analyzing sample groups by blood and bleach concentration, samples with BlueStar absent showed higher DNA yields than samples with BlueStar present at some bleach concentrations but not others (Figures 7-10). Therefore, the presence of BlueStar reagent may lower DNA yield in general but the reagent may have a chemical interaction with bleach that further interferes with DNA yields.

The default filter membrane for use with the M-Vac system is the PES membrane. This membrane is used for its DNA binding properties, but it is not the only membrane type available. Filter apparatuses with CN membranes can also be purchased and used with the M-Vac system. CN membranes have similar protein-binding properties and both membranes are available in a variety of pore sizes. The impact of filer membrane type can also be seen in Figure 3. The average DNA yield for PES membranes is lower than the average DNA yield for CN membranes for both experimental and control samples. Like the BlueStar results, analysis of blood and bleach concentrations showed that DNA yields for CN membranes were higher than DNA yields for PES membranes at some bleach concentrations but not others (Figures 7-10). The same conclusion could be made for the filter membranes, that CN membranes in general yield higher DNA concentrations than PES membranes, but the chemical interaction between the membrane types and bleach may additionally interfere with DNA yield.

Filtration product (filter vs. filtrate) impacted the DNA yield perhaps more than any other variable. The M-Vac system is designed to collect DNA on the filter membrane, and it is recommended that DNA isolation be performed from the filter membrane and not the remaining filtrate. Because bleach exhibits DNA-cleaving properties, it was hypothesized that some DNA fragments would pass through the 30 kDa membrane and remain in the filtrate. Many of the filtrate groups exhibited DNA concentrations greater than 0 ng/µL, indicating that some DNA

was allowed to pass through the membrane filter for those samples. However, the contrast between the DNA yields in the filters compared to the filtrates illustrated in Figure 3 is stark. Experimental filters averaged 0.022 ng/µL DNA while experimental filtrates averaged 0.003 ng/µL DNA. The difference between control filters and filtrates is even more dramatic, with control filters averaging 0.028 ng/µL DNA and control filtrates averaging 0.001 ng/µL DNA. It could be concluded that M-Vac filter membranes will yield more DNA than filtrates, regardless of bleach concentration, BlueStar presence, or filter membrane type.

4.2 Effects of Bleach

Bleach was found to have a profound effect on the forensic analysis of these samples via DNA degradation. Sodium hypochlorite (NaOCl), which is the chemical name for commercial bleach, reacts with DNA through oxidative damage. When DNA is exposed to bleach, the hydrogen bonds stabilizing the connection between base pairs are cleaved, resulting in "breaks" randomly throughout the double-stranded molecule. Because bleach cleaves DNA indiscriminately, meaning that cleavage sites are not targeted through any chemical forces, the number and size of the remaining DNA pieces cannot be predicted or modeled. Therefore, the concentration of bleach relative to the concentration of blood and reaction time are the two main factors that determine the extent to which DNA in a biological sample is degraded when exposed to bleach.

In this experiment, all combinations of the three blood and bleach concentrations (neat, 1:9, and 1:99) were tested. It was expected that as bleach concentration increased among samples all other variables consistent, DNA concentration would decrease. Results showed that this was not the case for any cohort of sample groups with variable bleach concentrations. Evidence for this can be readily seen in Table 4. For example, among groups with neat blood, filter, PES

membrane, and BlueStar present variables, the group with no bleach present predictably has the highest average DNA concentration at 0.05282 ng/µL, and the group with neat bleach predictably has the lowest average DNA concentration at 0.00342 ng/µL. However, the group with 1:9 bleach recovered a higher average concentration of DNA at 0.04402 ng/µL than the group with 1:99 bleach at 0.01439 ng/µL, despite 1:9 being a higher bleach concentration than 1:99. These results demonstrate the unpredictability of DNA degradation due to bleach exposure.

Furthermore, several experimental groups recovered higher average DNA concentrations than their respective control group, which was anticipated for the filtrate samples but unexpected for the filter samples. These groups are indicated with an asterisk (*) in Table 4. Among the 36 experimental groups from filter samples, 9 of the groups had higher average DNA concentrations than their respective control group. A plausible explanation for this result could be the inconsistencies in the M-Vac collection process, which were discussed earlier in section 4.1 Summary of Findings. Of the 36 experimental groups from filtrate samples, 11 of them had higher average DNA concentrations than their respective control group. This was expected for the filtrates because it was hypothesized that DNA exposed to bleach would cleave into pieces small enough to pass through the filter membrane and remain in the filtrate. With that hypothesis, it would stand to reason that filtrate groups with the highest concentration of bleach would reliably yield the highest concentration of DNA among groups with consistent blood concentration. No such trend is present in the data, which further demonstrates the erratic nature of bleach induced DNA degradation.

4.3 Effects of BlueStar

The addition of BlueStar forensic reagent had a notable impact on DNA recovery in this study. These results are consistent with prior research indicating that oxidative agents in BlueStar

reagent may degrade DNA in a similar fashion to bleach or interfere with forensic analysis (Larkin, 2012). As seen in Figure 3, the combined average DNA concentration of all groups with BlueStar absent is higher than the groups with BlueStar present, in both experimental and control groups. Among experimental groups, the combined average DNA concentration for BlueStar absent is 0.0148 ng/µL while the combined average DNA concentration for BlueStar present is 0.0100 ng/µL. This is a 48% increase in DNA recovery for samples without BlueStar. Among control groups, the combined average DNA concentration for BlueStar absent is 0.0164 ng/µL while the combined average DNA concentration for BlueStar present is 0.0135 ng/µL, which accounts for a 21% increase in DNA recovery for samples without BlueStar.

Table 4 further illustrates the impact of BlueStar. When comparing average DNA concentrations of filter sample groups with all variables identical except BlueStar presence, the group with BlueStar absent shows a higher DNA concentration than the group with BlueStar present in 22 out of the 24 group couples. The same examination of filtrate groups shows a different result – only half of the 24 group couples had a higher DNA concentration with BlueStar absent than with BlueStar present. However, as discussed in section 3.4.2 Analysis of experimental filtrate samples, the results from experimental filtrate groups are inconsistent and can be disregarded in this case.

4.4 Effect of Membrane Type

The type of membrane used, PES or CN, appears to have an impact on DNA recovery in this experiment as well. In Figure 3, the combined average DNA concentration for PES membranes of both experimental and control samples are significantly lower than the combined average DNA concentration for CN membranes. Differences in the chemical properties between the two membrane types may be responsible for these results. The CN membranes, composed of

a cellulose-based material, contain hydroxyl groups which are not present on the PES membranes. The hydroxyl group of cellulose-nitrate may heighten the CN membrane's DNA binding affinity. One caveat to the validity of these results, however, is that the portion of the membrane used in DNA isolation varied between the CN and PES membranes, which is discussed further in section 4.6 Methodological Limitations.

4.5 M-Vac Tubing Study

The interior of the tubing from the M-Vac line that delivers collected material to the collection bottle was swabbed and processed for DNA isolation and quantification in order to determine how much, if any, DNA is left behind in the tubing after the sampling process. The tubing, as well as the M-Vac head and collection bottles are marketed as single use materials, to reduce contamination in forensic investigations, however using fresh tubing for every sample may be costly for investigative agencies that employ the M-Vac system. Because the same M-Vac head and collection bottle were used for all samples in a group with identical blood, bleach, and BlueStar variables (the same materials used for all samples in groups A-1 and A-2 [Table 2]), only 26 total samples were taken of the M-Vac tubing. Of the 26 samples collected, all of them were found to have 0 ng/µL DNA except for two samples. The two samples that contained quantifiable amounts of DNA were samples 335 and 341 in Appendix A. The variables associated with sample 335 were BlueStar present, neat blood, and neat bleach, and the sample was found to have 0.00216 ng/µL DNA. The variables associated with sample 341 were BlueStar present, 1:99 blood, and neat bleach, and the sample was found to have 0.00098 ng/µL DNA. Both samples contained BlueStar reagent and neat bleach, but sample 335 had a much higher blood concentration than sample 341.

Although no hypotheses were made as to the results of this study, one would expect that the control samples with no bleach would have the highest concentration of DNA. However, the results show that no control samples contained DNA, and the only two samples that did contain quantifiable DNA were treated with the highest bleach concentration. Contamination during the sample collection or DNA isolation and quantification procedures could be responsible for these results. Therefore, no conclusions can be made in regard to this study and the practice of single-use M-Vac consumables in forensic settings should be continued until further research is conducted.

4.6 Methodological Limitations

Several factors throughout the sampling process and data analysis may have contributed to inconsistent or unreliable results in this project. In the sampling process, simulated cleaning of the bloodstain with bleach and kim wipes may have introduced unintended variability between samples. Although the author attempted to process each sample identically, it was difficult to control the procedure entirely. To what extent the bleach was allowed to react with the blood may be different from sample to sample due to involuntary changes in the amount of pressure applied to the bloodstain while rubbing in bleach. Similar to the simulated cleaning procedures, sampling with the M-Vac may not have been performed consistently across all samples. The author aimed to apply uniform pressure with the M-Vac head on the substrate during sampling but found it challenging to ensure the exact same technique was used each time. Variations likely occurred, with some sampling experiencing greater pressure from the M-Vac head, potentially increasing the amount of biological material that was loosened and collected. Additionally, in some instances, the M-Vac head may have been held over the bloodstain for a longer duration compared to others, which would further inflate the amount of biological material collected.

Furthermore, because the same M-Vac head and collection bottles were used for all samples with identical blood, bleach, and BlueStar variables, residual DNA left behind on the consumable materials may have introduced contamination between samples that were collected with the same supplies.

The two filter membranes used in this experiment, PES and CN, were not the same size which may have contributed to unreliable results in terms of data analysis. The PES membranes had a diameter of 50 mm while the CN membranes had a diameter of 45 mm. Despite this size difference, three 6 mm hole punches were taken from each membrane regardless of membrane diameter. Therefore, the portion of the CN membranes is larger than the portion of the PES membranes used for DNA isolation. This could distort the data so that the CN membranes appear to have higher DNA concentrations than they would if they were the same size as the PES membranes. To prevent this problem, the entire membrane or the same portion of each membrane should be used for DNA isolation in future experiments when using membranes with different sizes.

The low DNA quantities observed in many of the M-Vac samples present a challenge for reliable statistical analysis. Due to the presence of bleach in experimental samples, low DNA quantities were expected. However, many of the experimental DNA quantity values are outside of the range of the DNA standards used in the qPCR process. Quantitative PCR relies on a standard curve generated from the DNA standards with known concentrations to interpolate the DNA quantities of unknown samples. The lowest concentration of DNA standards used in this experiment was Std. 8 with a DNA concentration of 0.023 ng/µL (Table 3). 274 of the 307 DNA concentration values used for statistical analysis had a DNA concentration lower than Std. 8, meaning that for the majority of unknown samples, the DNA concentration values were

extrapolated from beyond the standard range. This introduces uncertainty as to the reliability of the data.

5. Conclusion

This goal of this project was to investigate the use of the M-Vac system for DNA collection from bloodstained substrates treated with bleach and to evaluate the effects of multiple variables, including three blood and bleach concentrations (neat, 1:9, and 1:99), the use of BlueStar reagent, the type of membrane used (PES vs. CN), and the DNA distribution between filters and filtrates. The study revealed insight into the effectiveness and limitations of DNA recovery with the M-Vac system in challenging forensic scenarios.

A key finding of this study is the substantial impact of bleach on DNA degradation, which proved to be disruptive to the quantity of DNA recovered from experimental samples. Bleach disrupts the double-stranded structure of DNA, breaking it into smaller fragments that are more difficult to retain on filter membranes like the ones used with the M-Vac system. Bleach was found to generally lower the DNA yield, however in some instances experimental groups were found to have higher DNA yields than their non-bleach control group counterpart. This was an expected result for filtrate samples because bleach was expected to fragment the DNA into smaller pieces that could flow through the filter and remain in the filtrate. However, it was not expected for filter samples. Slight inconsistencies and variances in the sampling process, particularly “cleaning” the bloodstain with bleach and the M-Vac collection procedure, may provide an explanation for why some experimental filter groups yielded higher DNA concentration than their non-bleach control group counterpart. Furthermore, the introduction of bleach caused extremely low DNA yields across all experimental samples, which creates a challenge for DNA quantification and calls into question the validity of the data. Because the DNA concentrations of many experimental samples were found to be outside of the range of the

qPCR standards, their values were extrapolated beyond the standard curve, which may compromise their accuracy and reliability for statistical analysis.

Another finding of this research is the differences between PES and CN membranes in terms of DNA recovery. CN membranes consistently yielded higher DNA concentrations than the PES membranes, though these results may be skewed due to the inconsistency in the portion of membrane used for DNA isolation. Both the PES and CN membranes had the same pore size, 0.45 µm, but the size of the membranes in diameter was not the same. The PES membranes were slightly larger at 50 mm in diameter than the CN membranes at 45 mm. The same amount of each membrane was taken for DNA isolation, however that amount of membrane made up a larger portion of the CN membrane than the PES membrane. Therefore, DNA concentration results for CN membranes might be artificially higher relative to the PES membrane results, than they would have been if the entire membrane or the same portion of membrane had been used for DNA isolation.

Another variable investigated in this experiment were the products of the filtration process – the filter and the filtrate. The M-Vac system is designed to concentrate all collected biological material on the filter membrane, and the filtrate is discarded. However, some biological material may pass through the filter and remain in the filtrate, especially in instances where a cleaning reagent like bleach has been used to obscure evidence, which could be a valuable source of evidence for investigators. It was hypothesized that bleach would cleave DNA molecules into smaller fragments that would be capable of passing through the 0.45 µm pores of the membranes, therefore the filtrate was kept and used for DNA isolation and quantification. Although quantifiable amounts of DNA were found in many sample groups of filtrate samples, the quantities of DNA found in filtrate samples were remarkably low compared to the filter

samples, and no consistent trends could be identified with regard to other variables involved (BlueStar presence or membrane type) therefore further statistical analysis was not performed. No conclusions can be made in regard to the value of using M-Vac filtrate as a source of DNA evidence in forensic investigations, except that quantifiable amounts of DNA may be found in the filtrate of M-Vac samples in some instances.

The final variable examined in this project was the presence or absence of BlueStar forensic reagent. BlueStar was incorporated into this project because of its potential to be used in forensic cases where bloodstains have been cleaned with a reagent like bleach that makes the bloodstains difficult for investigators to locate. The presence of BlueStar was found to lower the quantity of DNA recovered in experimental samples. This is consistent with existing literature stating that the oxidative properties of luminol-based reagents contribute to the degradation of DNA. These findings emphasize the trade-off between the value of BlueStar for crime scene visualization and its adverse effects on DNA evidence recovery. Further research should focus on strategies to mitigate these effects.

References

Adams, J. L., Rancourt, E. D., & Christensen, A. M. (2019). The Effect of Household Oxidizing Cleaners on Chemiluminescence of Blood using BlueStar® Forensic®†. *Journal of Forensic Sciences, 64*(3), 869–872. https://doi.org/10.1111/1556-4029.13914

Alberts, B. (2014). Chapter 3 – Proteins. In B. Alberts (Ed.) *Molecular Biology of the Cell, 6th edition.* (pp. 109 –172). Garland Science.

Ambers, A., Turnbough, M., Benjamin, R., King, J., & Budowle, B. (2014). Assessment of the role of DNA repair in damaged forensic samples. *International Journal of Legal Medicine,* 128, 913-921. https://doi.org/10.1007/s00414-014-1003-3

Applied Biosystems. (2018). *Quantifiler™ Human and Y Human Male DNA Quantification Kits User Guide.* Revision H.

Barbisin, M. & Shewale, J. G. (2013). Assessment of DNA Extracted from Forensic Samples Prior to Genotyping. In J. G. Shewale & R. H. Liu (Eds.) *Forensic DNA Analysis: Current Practices and Emerging Technologies. (*1st ed., pp. 101-128) CRC Press. https://doi-org.libproxy.uco.edu/10.1201/b15361

Butler, J. M. (2006). Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing. *Journal of Forensic Sciences, 51*(2), 253-265. https://doi.org/10.1111/j.1556-4029.2006.00046

Butler, J. M. (2012a). Chapter 2 – DNA Extraction Methods. In J. M. Butler (Ed.) *Advanced Topics in Forensic DNA Typing: Methodology.* (pp. 29-47) Academic Press. https://doi.org/10.1016/B978-0-12-374513-2.00002-6.

Butler, J. M. (2012b). Chapter 3 – DNA Quantitation. In J. M. Butler (Ed.) *Advanced Topics in Forensic DNA Typing: Methodology*. (pp. 49-67) Academic Press. https://doi.org/10.1016/B978-0-12-374513-2.00002-6.

Butler, J. M. (2012c). Chapter 4 – PCR Amplification: Capabilities and Cautions. In J. M. Butler (Ed.) *Advanced Topics in Forensic DNA Typing: Methodology*. (pp. 69-97) Academic Press. https://doi.org/10.1016/B978-0-12-374513-2.00002-6.

Caswell, K. L. (2014). *Comparison of M-Vac and Surface Swabs for Collecting Saliva and Touch DNA from Skin.* Available from ProQuest Dissertations & Theses Global.

Edler, C., Krebs, O., Gehl, A., Palatzke, K., Tiedemann, N., Schroder, A. S., & Klein, A. (2020). The effect of bleaching agents on the DNA analysis of bloodstains on different floor coverings. *International Journal of Legal Medicine,* 134, 921-927. https://doi.org/10.1007/s00414-020-02250-y

Garrett, A. D., Patlak, D. J., Gunn, L. E., Brodeur, A. N., & Grgicak, C. M. (2014). Exploring the Potential of a Wet-Vacuum Collection System for DNA Recovery. *Journal of Forensic Identification*, *64*(4), 429.

Harris, K. A., Thacker, C. R., Ballard, D., & Syndercombe Court, D. (2006). The effect of cleaning agents on the DNA analysis of blood stains deposited on different substrates. *International Congress Series,* 1288*:* 589-591. https://doi.org/10.1016/j.ics.2005.09.171

Hedman, J., Ågren J., & Ansell, R. (2015). Crime scene DNA sampling by wet-vacuum applying M-Vac. *Forensic Science International: Genetics Supplement Series*, Volume 5, e89-90. https://doi.org/10.1016/j.fsigss.2015.09.036.

Irion, P.R. (2020). Evaluating the Use of M-Vac® Wet Vacuum System to Recover DNA from Cotton Fabric. *Graduate Theses, Dissertations, and Problem Reports*. 7951. https://doi.org/10.33915/etd.7951

Jakovich, C. J. (2012). STR Analysis Following Latent Blood Detection by Luminol, Fluorescein, and BlueStar® Forensic. *Journal of Forensic Identification, 57*(2), 193-198.

Khan, P., Idrees, D., Moxley, M. A., Corbett, J. A., Ahmad, F., von Figura, G., Sly, W. S., Waheed, A., & Hassan, M. I. (2014). Luminol-based chemiluminescent signals: clinical and non-clinical application and future uses. *Applied biochemistry and biotechnology*, *173*(2), 333–355. https://doi.org/10.1007/s12010-014-0850-1

Kemp, R. A., & Allison, D. P. (2008). Oxidative Damage to DNA: Mechanisms and Implications. *Journal of Environmental Biochemistry*, 12(3), 111-123.

Larkin, A., van Oorschot, R.A.H., & Mitchell, R.J. (2012). The effect of luminol on DNA analysis. *Journal of Forensic Sciences, 57*(1), 200-203.

Li, R. C., Harris, & H. A. (2003). Using Hydrophilic Adhesive Tape for Collection of Evidence for Forensic DNA Analysis. *Journal of Forensic Sciences, 48*(6), 1318-1321.

McLamb, J. M., & Kavlick, M. F. (2020, February 14-22) *Practical applications of a wet-vacuum DNA collection system* [Poster presentation]. 72nd AAFS Annual Scientific Meeting, Anaheim, CA, United States of America.

McLamb, J. M., Adams, L. D., & Kavlick, M. F. (2020). Comparison of the M-Vac® Wet-Vacuum-Based Collection Method to a Wet-Swabbing Method for DNA Recovery on

Diluted Bloodstained Substrates*, †, ‡. *Journal of Forensic Sciences*, *65*(6), 1828–1834. https://doi.org/10.1111/1556-4029.14508

Mulligan, C. M., Kauffman, S. R., & Quarino, L. (2011). The Utility of Polyester and Cotton Swabbing Substrates for the Removal of Cellular Material from Surfaces. *Journal of Forensic Sciences, 56*(2), 485-490. https://doi.org/10.1111/j.1556-4029.2010.01659.x

M-Vac Systems®, Inc. (n.d.) *How Does the M-Vac Work?* https://www.m-vac.com/why-mvac/how-it-works#

M-Vac Systems®, Inc. (n.d.) *Research.* https://www.m-vac.com/why-mvac/research

OpenAI. (2024). *ChatGPT (November 2024 version).* [AI model]. https://openai.com. *Note: ChatGPT was used for syntax refinement and editing assistance. The content and interpretations remain the sole responsibility of the author.*

Passi, N., Garg, R. K., Yadav, M., Singh, R. S., & Kharoshah, M. A. (2012). Effect of lumiol and bleaching agent on the serological and DNA analysis from bloodstain. *Egyptian Journal of Forensic Science*, 2, 54-61. https://doi.org/10.1016/j.ejfs.2012.04.003

Patel, G., & Hopwood, A. (2013). An evaluation of luminol formulations and their effect on DNA profiling. *International journal of legal medicine*, *127*(4), 723–729. https://doi.org/10.1007/s00414-012-0800-9

Promega. (2016). *DNA IQ™ System – Database Protocol.* Retrieved from https://www.promega.com/-/media/files/resources/protocols/technical-bulletins/101/dna-iq-system-database-protocol.pdf

Promega. (2020). *PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers*. Retrieved from https://www.promega.com/~/media/files/resources/protocols/technical-manuals/101/powerplex-fusion-system-protocol.pdf

Shewale, J. G., Liwei, Q. & Calandro, L. M. (2013). Principles, Practice, and Evolution of Capillary Electrophoresis as a Tool for Forensic DNA Analysis. In J. G. Shewale & R. H. Liu (Eds.) *Forensic DNA Analysis: Current Practices and Emerging Technologies.* (1st ed., pp. 131-162) CRC Press. https://doi-org.libproxy.uco.edu/10.1201/b15361

Stouder, S. L., Reubush, K. J., Hobson, D. L., & Smith, J. L. (2001). Trace Evidence Scrapings: A Valuable Source of DNA? *Forensic Science Communications, 3*(4), 1-6.

Stray, J. E., Liu, J. Y., Brevnov, M. G., & Shewale, J. G. (2013). Extraction of DNA from Forensic Biological Samples for Genotyping. In J. G. Shewale & R. H. Liu (Eds.) *Forensic DNA Analysis: Current Practices and Emerging Technologies*. (1st ed., pp. 39-64) CRC Press. https://doi-org.libproxy.uco.edu/10.1201/b15361

Sweet, D., Lorente, M, Lorente, J. A., Valenzuela, A., & Villanueva, E. (1997). An improved Method to Recover Salia from Human Skin: The Double Swab Technique. *Journal of Forensic Sciences, 42* (2), 320-332.

Thabet, H. Z., Ghandour, N. M., & Salama, R. H. (2018). Effect of Some Cleaning Products on Blood DNA Retrieval from Cloth. *Egyptian Journal of Forensic Science and Applied Toxicology, 18*(2), 53-66.

Verdon, T. J., Mitchell, R. J., & van Oorschot, R. A. (2014). Swabs as DNA collection devices for sampling different biological materials from different substrates. *Journal of Forensic Sciences, 59*(4), 1080–1089. https://doi.org/10.1111/1556-4029.12427

Vickar, T., Bache, K., Daniel B., & Frascione, N. (2018). The use of the M-Vac® wet-vacuum system as a method for DNA recovery. *Science & Justice.* https://doi.org/10.1016/j.scijus.2018.01.003

Wander, M. J. (2014). *An Investigation of Touch DNA Collection Methods from Clothing: Traditional Cutting Techniques Versus a Wet Vacuum System*. Available from ProQuest Central; ProQuest Dissertations & Theses Global.

Williams, S. E. (2014). *Recovery of Salivary DNA from the Skin after Showering, Using the M-Vac*®. Available from ProQuest Dissertations & Theses Global.

Appendix A: DNA Concentrations

#	Sample Type	Membrane Type	BlueStar Presence	Blood Conc.	Bleach Conc.	DNA Conc. (ng/µL)	Average Conc.	Standard Deviation
1	Filtrate	PES	Absent	Neat	Neat	0.0091	0.00303	0.00525
2	Filtrate	PES	Absent	Neat	Neat	0		
3	Filtrate	PES	Absent	Neat	Neat	0		
4	Filtrate	CN	Absent	Neat	Neat	0	0.00272	0.00471
5	Filtrate	CN	Absent	Neat	Neat	0		
6	Filtrate	CN	Absent	Neat	Neat	0.00815		
7*	Filtrate	PES	Absent	Neat	1:9	229.21981	0.00000	0.00000
8	Filtrate	PES	Absent	Neat	1:9	0		
9	Filtrate	PES	Absent	Neat	1:9	0		
10	Filtrate	CN	Absent	Neat	1:9	0.00177	0.00059	0.00102
11	Filtrate	CN	Absent	Neat	1:9	0		
12	Filtrate	CN	Absent	Neat	1:9	0		
13	Filtrate	PES	Absent	Neat	1:99	0	0.00066	0.00115
14	Filtrate	PES	Absent	Neat	1:99	0.00199		
15	Filtrate	PES	Absent	Neat	1:99	0		
16	Filtrate	CN	Absent	Neat	1:99	0	0.00052	0.00089
17	Filtrate	CN	Absent	Neat	1:99	0		
18	Filtrate	CN	Absent	Neat	1:99	0.00155		
19	Filtrate	PES	Absent	1:9	Neat	0	0.00000	0.00000
20	Filtrate	PES	Absent	1:9	Neat	0		
21	Filtrate	PES	Absent	1:9	Neat	0		
22	Filtrate	CN	Absent	1:9	Neat	0	0.00000	0.00000
23*	Filtrate	CN	Absent	1:9	Neat	45,580.23		
24	Filtrate	CN	Absent	1:9	Neat	0		
25	Filtrate	PES	Absent	1:9	1:9	0	0.00000	0.00000
26	Filtrate	PES	Absent	1:9	1:9	0		
27	Filtrate	PES	Absent	1:9	1:9	0		
28	Filtrate	CN	Absent	1:9	1:9	0	0.00000	0.00000
29	Filtrate	CN	Absent	1:9	1:9	0		
30	Filtrate	CN	Absent	1:9	1:9	0		
31	Filtrate	PES	Absent	1:9	1:99	0.00416	0.00139	0.00240
33	Filtrate	PES	Absent	1:9	1:99	0		
34	Filtrate	PES	Absent	1:9	1:99	0		
35	Filtrate	CN	Absent	1:9	1:99	0	0.00000	0.00000
36	Filtrate	CN	Absent	1:9	1:99	0		
37	Filtrate	CN	Absent	1:9	1:99	0		
38*	Filtrate	PES	Absent	1:99	Neat	61.1928	0.00237	0.00334
39	Filtrate	PES	Absent	1:99	Neat	0		
40	Filtrate	PES	Absent	1:99	Neat	0.00473		
41	Filtrate	CN	Absent	1:99	Neat	0.00384	0.00128	0.00222
42	Filtrate	CN	Absent	1:99	Neat	0		
43	Filtrate	CN	Absent	1:99	Neat	0		
44	Filtrate	PES	Absent	1:99	1:9	0	0.00000	0.00000
45	Filtrate	PES	Absent	1:99	1:9	0		
46	Filtrate	PES	Absent	1:99	1:9	0		

47	Filtrate	CN	Absent	1:99	1:9	0.00524	0.00343	0.00297
48	Filtrate	CN	Absent	1:99	1:9	0.00504		
49	Filtrate	CN	Absent	1:99	1:9	0		
50	Filtrate	PES	Absent	1:99	1:99	0	0.00054	0.00093
51	Filtrate	PES	Absent	1:99	1:99	0.00161		
52	Filtrate	PES	Absent	1:99	1:99	0		
53	Filtrate	CN	Absent	1:99	1:99	0	0.00000	0.00000
54	Filtrate	CN	Absent	1:99	1:99	0		
55	Filtrate	CN	Absent	1:99	1:99	0		
56	Filtrate	PES	Present	Neat	Neat	0.00517	0.00172	0.00298
57	Filtrate	PES	Present	Neat	Neat	0		
58	Filtrate	PES	Present	Neat	Neat	0		
59	Filtrate	CN	Present	Neat	Neat	0	0.00000	0.00000
60	Filtrate	CN	Present	Neat	Neat	0		
61	Filtrate	CN	Present	Neat	Neat	0		
62	Filtrate	PES	Present	Neat	1:9	0.00155	0.00052	0.00089
63	Filtrate	PES	Present	Neat	1:9	0		
65	Filtrate	PES	Present	Neat	1:9	0		
66	Filtrate	CN	Present	Neat	1:9	0.0052	0.00238	0.00263
67	Filtrate	CN	Present	Neat	1:9	0.00195		
68	Filtrate	CN	Present	Neat	1:9	0		
69	Filtrate	PES	Present	Neat	1:99	0.00442	0.00833	0.00358
70	Filtrate	PES	Present	Neat	1:99	0.00911		
71	Filtrate	PES	Present	Neat	1:99	0.01146		
72	Filtrate	CN	Present	Neat	1:99	0.18865	0.06847	0.10410
73	Filtrate	CN	Present	Neat	1:99	0.01015		
74	Filtrate	CN	Present	Neat	1:99	0.0066		
75	Filtrate	PES	Present	1:9	Neat	0.01547	0.00788	0.00774
76	Filtrate	PES	Present	1:9	Neat	0		
77	Filtrate	PES	Present	1:9	Neat	0.00816		
78	Filtrate	CN	Present	1:9	Neat	0	0.00000	0.00000
79	Filtrate	CN	Present	1:9	Neat	0		
80	Filtrate	CN	Present	1:9	Neat	0		
81	Filtrate	PES	Present	1:9	1:9	0	0.00000	0.00000
82	Filtrate	PES	Present	1:9	1:9	0		
83	Filtrate	PES	Present	1:9	1:9	0		
84	Filtrate	CN	Present	1:9	1:9	0.00181	0.00182	0.00183
85	Filtrate	CN	Present	1:9	1:9	0		
86	Filtrate	CN	Present	1:9	1:9	0.00366		
87	Filtrate	PES	Present	1:9	1:99	0	0.00000	0.00000
88	Filtrate	PES	Present	1:9	1:99	0		
89	Filtrate	PES	Present	1:9	1:99	0		
90	Filtrate	CN	Present	1:9	1:99	0.00214	0.00140	0.00121
91	Filtrate	CN	Present	1:9	1:99	0.00205		
92	Filtrate	CN	Present	1:9	1:99	0		
93	Filtrate	PES	Present	1:99	Neat	0	0.00000	0.00000
94	Filtrate	PES	Present	1:99	Neat	0		
95	Filtrate	PES	Present	1:99	Neat	0		
97	Filtrate	CN	Present	1:99	Neat	0.00098	0.00033	0.00057

98	Filtrate	CN	Present	1:99	Neat	0		
99	Filtrate	CN	Present	1:99	Neat	0		
100	Filtrate	PES	Present	1:99	1:9	0	0.00000	0.00000
101	Filtrate	PES	Present	1:99	1:9	0		
102	Filtrate	PES	Present	1:99	1:9	0		
103	Filtrate	CN	Present	1:99	1:9	0	0.00000	0.00000
104	Filtrate	CN	Present	1:99	1:9	0		
105	Filtrate	CN	Present	1:99	1:9	0		
106	Filtrate	PES	Present	1:99	1:99	0	0.00035	0.00060
107	Filtrate	PES	Present	1:99	1:99	0		
108	Filtrate	PES	Present	1:99	1:99	0.00104		
109	Filtrate	CN	Present	1:99	1:99	0.00112	0.00107	0.00104
110	Filtrate	CN	Present	1:99	1:99	0		
111	Filtrate	CN	Present	1:99	1:99	0.00208		
112	Filtrate	PES	Present	Neat	None	0	0.00135	0.00118
113	Filtrate	PES	Present	Neat	None	0.00214		
114	Filtrate	PES	Present	Neat	None	0.00192		
115	Filtrate	CN	Present	Neat	None	0.00187	0.00276	0.00329
116	Filtrate	CN	Present	Neat	None	0		
117	Filtrate	CN	Present	Neat	None	0.0064		
118	Filtrate	PES	Absent	Neat	None	0	0.00169	0.00156
119	Filtrate	PES	Absent	Neat	None	0.00202		
120	Filtrate	PES	Absent	Neat	None	0.00306		
121	Filtrate	CN	Absent	Neat	None	0.00205	0.00407	0.00335
122	Filtrate	CN	Absent	Neat	None	0.00222		
123	Filtrate	CN	Absent	Neat	None	0.00794		
124	Filtrate	PES	Present	1:9	None	0	0.00297	0.00301
125	Filtrate	PES	Present	1:9	None	0.0029		
126	Filtrate	PES	Present	1:9	None	0.00601		
127	Filtrate	CN	Present	1:9	None	0	0.00068	0.00117
129	Filtrate	CN	Present	1:9	None	0		
130	Filtrate	CN	Present	1:9	None	0.00203		
131	Filtrate	PES	Absent	1:9	None	0		
132	Filtrate	PES	Absent	1:9	None	0		
133	Filtrate	PES	Absent	1:9	None	0	0.00000	0.00000
134	Filtrate	CN	Absent	1:9	None	0	0.00154	0.00266
135	Filtrate	CN	Absent	1:9	None	0		
136	Filtrate	CN	Absent	1:9	None	0.00461		
137	Filtrate	PES	Present	1:99	None	0.00106	0.00035	0.00061
138	Filtrate	PES	Present	1:99	None	0		
139	Filtrate	PES	Present	1:99	None	0		
140	Filtrate	CN	Present	1:99	None	0	0.00146	0.00149
141	Filtrate	CN	Present	1:99	None	0.0014		
142	Filtrate	CN	Present	1:99	None	0.00297		
143	Filtrate	PES	Absent	1:99	None	0	0.00000	0.00000
144	Filtrate	PES	Absent	1:99	None	0		
145	Filtrate	PES	Absent	1:99	None	0		
146	Filtrate	CN	Absent	1:99	None	0.00217	0.00072	0.00125
147	Filtrate	CN	Absent	1:99	None	0		

148	Filtrate	CN	Absent	1:99	None	0		
149	Filtrate	PES	Present	None	None	0	0.00510	0.00760
150	Filtrate	PES	Present	None	None	0.00146		
151	Filtrate	PES	Present	None	None	0.01384		
152	Filtrate	CN	Present	None	None	0	0.00000	0.00000
153	Filtrate	CN	Present	None	None	0		
154	Filtrate	CN	Present	None	None	0		
155	Filtrate	PES	Absent	None	None	0	0.00000	0.00000
156*	Filtrate	PES	Absent	None	None	1783332.096		
157	Filtrate	PES	Absent	None	None	0		
158*	Filtrate	CN	Absent	None	None	45340144.91	0.00000	0.00000
159	Filtrate	CN	Absent	None	None	0		
160	Filtrate	CN	Absent	None	None	0		
162	Filter	PES	Absent	Neat	Neat	0.00199	0.00400	0.00221
163	Filter	PES	Absent	Neat	Neat	0.00637		
164	Filter	PES	Absent	Neat	Neat	0.00363		
165	Filter	CN	Absent	Neat	Neat	0.00801	0.00414	0.00360
166	Filter	CN	Absent	Neat	Neat	0.00352		
167	Filter	CN	Absent	Neat	Neat	0.0009		
168	Filter	PES	Absent	Neat	1:9	0.05295	0.05668	0.01500
169	Filter	PES	Absent	Neat	1:9	0.0732		
170	Filter	PES	Absent	Neat	1:9	0.0439		
171	Filter	CN	Absent	Neat	1:9	0.17178	0.16042	0.01810
172	Filter	CN	Absent	Neat	1:9	0.13955		
173	Filter	CN	Absent	Neat	1:9	0.16994		
174	Filter	PES	Absent	Neat	1:99	0.06905	0.11415	0.04036
175	Filter	PES	Absent	Neat	1:99	0.1265		
176	Filter	PES	Absent	Neat	1:99	0.14689		
177	Filter	CN	Absent	Neat	1:99	0.14664	0.17097	0.03985
178	Filter	CN	Absent	Neat	1:99	0.21696		
179	Filter	CN	Absent	Neat	1:99	0.14932		
180	Filter	PES	Absent	1:9	Neat	0	0.00000	0.00000
181	Filter	PES	Absent	1:9	Neat	0		
182	Filter	PES	Absent	1:9	Neat	0		
183	Filter	CN	Absent	1:9	Neat	0	0.00078	0.00135
184	Filter	CN	Absent	1:9	Neat	0		
185	Filter	CN	Absent	1:9	Neat	0.00233		
186	Filter	PES	Absent	1:9	1:9	0	0.00166	0.00288
187	Filter	PES	Absent	1:9	1:9	0.00498		
188	Filter	PES	Absent	1:9	1:9	0		
189	Filter	CN	Absent	1:9	1:9	0	0.00034	0.00058
190	Filter	CN	Absent	1:9	1:9	0.00101		
191	Filter	CN	Absent	1:9	1:9	0		
192	Filter	PES	Absent	1:9	1:99	0	0.00083	0.00144
194	Filter	PES	Absent	1:9	1:99	0		
195	Filter	PES	Absent	1:9	1:99	0.0025		
196	Filter	CN	Absent	1:9	1:99	0	0.00072	0.00125
197	Filter	CN	Absent	1:9	1:99	0.00216		
198	Filter	CN	Absent	1:9	1:99	0		

199	Filter	PES	Absent	1:99	Neat	0	0.00000	0.00000
200	Filter	PES	Absent	1:99	Neat	0		
201	Filter	PES	Absent	1:99	Neat	0		
202	Filter	CN	Absent	1:99	Neat	0	0.00000	0.00000
203	Filter	CN	Absent	1:99	Neat	0		
204	Filter	CN	Absent	1:99	Neat	0		
205	Filter	PES	Absent	1:99	1:9	0	0.00069	0.00120
206	Filter	PES	Absent	1:99	1:9	0.00208		
207	Filter	PES	Absent	1:99	1:9	0		
208	Filter	CN	Absent	1:99	1:9	0	0.00157	0.00271
209	Filter	CN	Absent	1:99	1:9	0.0047		
210	Filter	CN	Absent	1:99	1:9	0		
211	Filter	PES	Absent	1:99	1:99	0	0.00045	0.00077
212	Filter	PES	Absent	1:99	1:99	0		
213	Filter	PES	Absent	1:99	1:99	0.00134		
214	Filter	CN	Absent	1:99	1:99	0	0.00000	0.00000
215	Filter	CN	Absent	1:99	1:99	0		
216	Filter	CN	Absent	1:99	1:99	0		
217	Filter	PES	Present	Neat	Neat	0.00211	0.00342	0.00132
218	Filter	PES	Present	Neat	Neat	0.0034		
219	Filter	PES	Present	Neat	Neat	0.00475		
220	Filter	CN	Present	Neat	Neat	0.04964	0.02101	0.02480
221	Filter	CN	Present	Neat	Neat	0.00706		
222	Filter	CN	Present	Neat	Neat	0.00633		
223	Filter	PES	Present	Neat	1:9	0.09827	0.04402	0.04817
224	Filter	PES	Present	Neat	1:9	0.02756		
226	Filter	PES	Present	Neat	1:9	0.00624		
227	Filter	CN	Present	Neat	1:9	0.02679	0.08128	0.07382
228	Filter	CN	Present	Neat	1:9	0.16529		
229	Filter	CN	Present	Neat	1:9	0.05177		
230	Filter	PES	Present	Neat	1:99	0.01744	0.01439	0.00470
231	Filter	PES	Present	Neat	1:99	0.00898		
232	Filter	PES	Present	Neat	1:99	0.01676		
233	Filter	CN	Present	Neat	1:99	0.1119	0.10092	0.04388
234	Filter	CN	Present	Neat	1:99	0.0526		
235	Filter	CN	Present	Neat	1:99	0.13827		
236	Filter	PES	Present	1:9	Neat	0	0.00000	0.00000
237	Filter	PES	Present	1:9	Neat	0		
238	Filter	PES	Present	1:9	Neat	0		
239	Filter	CN	Present	1:9	Neat	0	0.00000	0.00000
240	Filter	CN	Present	1:9	Neat	0		
241	Filter	CN	Present	1:9	Neat	0		
242	Filter	PES	Present	1:9	1:9	0	0.00000	0.00000
243	Filter	PES	Present	1:9	1:9	0		
244	Filter	PES	Present	1:9	1:9	0		
245	Filter	CN	Present	1:9	1:9	0	0.00000	0.00000
246	Filter	CN	Present	1:9	1:9	0		
247	Filter	CN	Present	1:9	1:9	0		
248	Filter	PES	Present	1:9	1:99	0	0.00050	0.00086

249	Filter	PES	Present	1:9	1:99	0		
250	Filter	PES	Present	1:9	1:99	0.00149		
251	Filter	CN	Present	1:9	1:99	0	0.00000	0.00000
252	Filter	CN	Present	1:9	1:99	0		
253	Filter	CN	Present	1:9	1:99	0		
254	Filter	PES	Present	1:99	Neat	0	0.00000	0.00000
255	Filter	PES	Present	1:99	Neat	0		
256	Filter	PES	Present	1:99	Neat	0		
258	Filter	CN	Present	1:99	Neat	0	0.00000	0.00000
259	Filter	CN	Present	1:99	Neat	0		
260	Filter	CN	Present	1:99	Neat	0		
261	Filter	PES	Present	1:99	1:9	0	0.00047	0.00082
262	Filter	PES	Present	1:99	1:9	0		
263	Filter	PES	Present	1:99	1:9	0.00142		
264	Filter	CN	Present	1:99	1:9	0	0.00000	0.00000
265	Filter	CN	Present	1:99	1:9	0		
266	Filter	CN	Present	1:99	1:9	0		
267	Filter	PES	Present	1:99	1:99	0.00307	0.00102	0.00177
268	Filter	PES	Present	1:99	1:99	0		
269	Filter	PES	Present	1:99	1:99	0		
270	Filter	CN	Present	1:99	1:99	0	0.00000	0.00000
271	Filter	CN	Present	1:99	1:99	0		
272	Filter	CN	Present	1:99	1:99	0		
273	Filter	PES	Present	Neat	None	0.04284	0.05282	0.03378
274	Filter	PES	Present	Neat	None	0.09046		
275	Filter	PES	Present	Neat	None	0.02515		
276	Filter	CN	Present	Neat	None	0.04296	0.09902	0.10157
277	Filter	CN	Present	Neat	None	0.21626		
278	Filter	CN	Present	Neat	None	0.03784		
279	Filter	PES	Absent	Neat	None	0.13045	0.05806	0.06313
280	Filter	PES	Absent	Neat	None	0.01444		
281	Filter	PES	Absent	Neat	None	0.02928		
282	Filter	CN	Absent	Neat	None	0.09222		
283	Filter	CN	Absent	Neat	None	0.1476		
284	Filter	CN	Absent	Neat	None	0.11064	0.11682	0.02820
285	Filter	PES	Present	1:9	None	0	0.00000	0.00000
286	Filter	PES	Present	1:9	None	0		
287	Filter	PES	Present	1:9	None	0		
288	Filter	CN	Present	1:9	None	0.00138	0.00046	0.00080
290	Filter	CN	Present	1:9	None	0		
291	Filter	CN	Present	1:9	None	0		
292	Filter	PES	Absent	1:9	None	0.00512	0.00696	0.00162
293	Filter	PES	Absent	1:9	None	0.00759		
294	Filter	PES	Absent	1:9	None	0.00817		
295	Filter	CN	Absent	1:9	None	0.00584	0.00681	0.00178
296	Filter	CN	Absent	1:9	None	0.00887		
297	Filter	CN	Absent	1:9	None	0.00573		
298	Filter	PES	Present	1:99	None	0	0.00000	0.00000
299	Filter	PES	Present	1:99	None	0		

300	Filter	PES	Present	1:99	None	0		
301	Filter	CN	Present	1:99	None	0	0.00000	0.00000
302	Filter	CN	Present	1:99	None	0		
303	Filter	CN	Present	1:99	None	0		
304	Filter	PES	Absent	1:99	None	0	0.00000	0.00000
305	Filter	PES	Absent	1:99	None	0		
306	Filter	PES	Absent	1:99	None	0		
307	Filter	CN	Absent	1:99	None	0	0.00000	0.00000
308	Filter	CN	Absent	1:99	None	0		
309	Filter	CN	Absent	1:99	None	0		
310	Filter	PES	Present	None	None	0	0.00000	0.00000
311	Filter	PES	Present	None	None	0		
312	Filter	PES	Present	None	None	0		
313	Filter	CN	Present	None	None	0	0.00000	0.00000
314	Filter	CN	Present	None	None	0		
315	Filter	CN	Present	None	None	0		
316	Filter	PES	Absent	None	None	0	0.00000	0.00000
317	Filter	PES	Absent	None	None	0		
318	Filter	PES	Absent	None	None	0		
319	Filter	CN	Absent	None	None	0	0.00000	0.00000
320	Filter	CN	Absent	None	None	0		
321	Filter	CN	Absent	None	None	0		
323	LB	-	-	Neat	-	0.38974	-	-
324	LB	-	-	1:9	-	0.29635	-	-
325	LB	-	-	1:99	-	0.10449	-	-
326	Swab	-	Absent	Neat	Neat	0	-	-
327	Swab	-	Absent	Neat	1:9	0	-	-
328	Swab	-	Absent	Neat	1:99	0	-	-
329	Swab	-	Absent	1:9	Neat	0	-	-
330	Swab	-	Absent	1:9	1:9	0	-	-
331	Swab	-	Absent	1:9	1:99	0	-	-
332	Swab	-	Absent	1:99	Neat	0	-	-
333	Swab	-	Absent	1:99	1:9	0	-	-
334	Swab	-	Absent	1:99	1:99	0	-	-
335	Swab	-	Present	Neat	Neat	0.00216	-	-
336	Swab	-	Present	Neat	1:9	0	-	-
337	Swab	-	Present	Neat	1:99	0	-	-
338	Swab	-	Present	1:9	Neat	0	-	-
339	Swab	-	Present	1:9	1:9	0	-	-
340	Swab	-	Present	1:9	1:99	0	-	-
341	Swab	-	Present	1:99	Neat	0.00098	-	-
342	Swab	-	Present	1:99	1:9	0	-	-
343	Swab	-	Present	1:99	1:99	0	-	-
344	Swab	-	Present	Neat	None	0	-	-
345	Swab	-	Absent	Neat	None	0	-	-
346	Swab	-	Present	1:9	None	0	-	-
347	Swab	-	Absent	1:9	None	0	-	-
348	Swab	-	Present	1:99	None	0	-	-
349	Swab	-	Absent	1:99	None	0	-	-

350	Swab	-	Present	None	None	0	-	-
351	Swab	-	Absent	None	None	0	-	-
352	Buccal Swab	-	-	-	-	7.43904	-	-
353	Buccal Swab	-	-	-	-	7.05778	-	-

* Outliers. Not used in statistical analysis.