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EVALUATING THE EFFICIENCY OF LAUNDRY SANITIZERS ON THE REMOVAL AND TRANSFER OF DNA IN A WASHING MAHCINE

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EVALUATING THE EFFICIENCY OF LAUNDRY SANITIZERS ON THE REMOVAL AND TRANSFER OF DNA IN A WASHING MACHINE

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Abstract

DNA persistence and transfer has been a topic of much research and study over the last several years in the forensic science field. One interesting subfield that has been investigated is the field of laundered fabrics. It has been discovered that DNA is recoverable from laundered fabrics. The sources include not only biological stains but also transfer during wear. Remarkably, DNA is able to persist through the washing process, while also capable of transferring onto other pieces of fabric during the washing process. This means a piece of evidence with one person's DNA could transfer DNA onto a piece of fabric that was initially free of DNA. Many variables affect the persistence and transfer of DNA during the washing process including the temperature of the water, hand-washing versus machine-washing, and the cleaning products used in the washing process. One laundry product that has increased use in the last several years is the laundry sanitizer, which aims to eliminate bacteria and viruses from clothing rather than dirt. The laundry sanitizer is rarely mentioned in the literature and requires more research.

This thesis project focuses on two main specific aims. Specific Aim 1 is focused on the persistence of DNA from wear during the washing process. Six different laundry products were tested, along with two separate collection methods of the wet vacuum, also known as the M-Vac™, and conventional swabbing. Lastly, the set of directions used for the laundry sanitizer was also evaluated. Laundry sanitizers often have two sets of directions: one utilizing a pre-soak option and the other utilizing the sanitizer as part of the rinse cycle. Specific Aim 2 is focused on the transfer of DNA from a washcloth spotted with blood to another pristine washcloth. The same products, except for the dry-cleaning products, were tested with the same two collection methods. The last variable tested was the blood input. Half of the washcloths were spotted with three drops of blood, and the other half were spotted with one drop of blood.

To account for the high frequency of zero values, a two-part hurdle model was employed. This approach utilized a logistic regression to model the probability of occurrence and a linear regression on log-transformed data to analyze the remaining non-zero concentrations. It was seen that laundry detergent tended to have a statistically significant result on DNA concentrations. The laundry products were sometimes found to have significantly higher DNA concentrations than the low control of bleach. The collection method had some contradictory findings. In Specific Aim 1, it was seen that the M-Vac™ was associated with lower DNA concentrations than the bleach control, while in Specific Aim 2 it was seen that the M-Vac™ was associated with higher concentrations than the bleach control. Neither the blood input nor the set of directions used was ever found to be statistically significant. Bacterial colonies were also assessed in Specific Aim 1 with the shirts, and it was found that only some products (OxiClean™, Silver Satin 2+, and Lysol™) never had any colonies grow. A lack of colony growth was also associated with the rinse cycle set of directions.

DNA profiles of select samples were assessed to see the quality and quantity of information that could be obtained from these samples. In terms of quality, the electropherograms were for the most part clear single source profiles with few artifacts present. There was one profile that was a partial profile with significant allelic dropout. When the electropherograms were compared with the known profiles of the participants, it was found that both the participants were able to be excluded from the sample DNA profiles. It was found that a member of the household where the washing machine was located could not be excluded from the sample DNA profiles. This was despite extensive cleaning protocols before the project began and between each sample to avoid such occurrences.

Altogether, there were several interesting findings from this study in terms of the importance of laundry sanitizers on the persistence and transfer of DNA in a washing machine. It also revealed some intriguing trends about the usage of the M-Vac™ on porous fabrics. However, this study would benefit from an increased number of replicates to confirm the trends identified and perhaps parse out other trends not identified in this study. Furthermore, more troubleshooting with the cleaning protocol should be conducted or perhaps other locations for washing machines explored.

1. Introduction

In forensic biology, a topic of research that has been heavily studied in the last few years has been transfer and persistence of DNA (van Oorschot et al., 2021). DNA transfers much easier than imagined before DNA kits became more sensitive and revealed this information. It is imperative for the forensic science field to know as much about DNA transfer as possible to aid them in making decisions during interpretation analysis and while testifying in front of the jury. Forensic scientists are increasingly being asked to answer questions about activity levels during the crime. Activity levels refer to the question of how a particular DNA profile appeared on the evidence or the crime scene (Kokshoorn et al., 2017). Most of the time, the question that is being asked is whether a profile is there through direct contact or through indirect transfer from another intermediate surface. Having information about one hypothesis or the other could severely affect the outcome of the case.

A crucial piece of evidence could come from the clothing that is found at the scene or was worn by either the victim or suspect. However, it is also fairly easy to try and destroy that evidence by washing the clothing, such as putting it in the washing machine. It has been shown that DNA is able to not only persist through the washing machine but also transfer from one piece of clothing to another while in the washing machine (Ruan et al., 2018; Voskoboinik et al., 2018). Certain laundry products have been found to influence the persistence of DNA on clothing and therefore being more or less likely to extract a usable DNA profile from the clothing even after being washed (Thabet et al., 2018). A new laundry product has emerged on the market and grown in popularity since the COVID-19 pandemic, the laundry sanitizer. Despite growing in popularity, laundry sanitizer has barely been used in DNA persistence and transfer research. It has mainly been studied in terms of it removing bacteria and viruses from clothing (Farcas et al.,

2019; Khalid Ijaz et al., 2022). This lack of information could be detrimental to forensic analysts if there is a case involving this laundry product.

Additionally, the collection method is also an important variable to study. Porous objects have long been known to be difficult to collect DNA from, as the DNA permeates the object itself. Conventional methods such as swabbing may not yield a lot of DNA. A new collection method has been developed for forensic use, the microbial wet vacuum, or the M-Vac™. It has been seen to be useful for increasing the amount of DNA collected from porous subjects (Blackmore et al., 2024; Vickar et al., 2018). This project will also work to evaluate the effectiveness of the M-Vac™.

This thesis project's goal is to study the effect of laundry sanitizer on the persistence and transfer of DNA in a washing machine. After the project is completed, a deeper understanding of laundry sanitizers will be reached and added to the collective pool of information that forensic analysts rely on. In essence, this project aims to increase knowledge of DNA transfer and the importance of collection methods, thereby assisting forensic analysts in the field. Furthermore, a comparison of the M-Vac™ and conventional collection methods will be conducted to see if the M-Vac™ is truly superior in collecting DNA from porous substrates. This could be important news for forensic analysts and lead to this technique being adopted in the field. It is hypothesized that laundry sanitizers will affect the amount of DNA recovered from washed clothing since other laundry products have been shown to do so already. Additionally, it is hypothesized that the M-Vac™ will outperform the conventional collection methods, since many other porous substrates have been tested and showed promising results.

2. Literature Review

2.1 DNA in Forensic Science

DNA evidence is a very important piece of evidence in forensic science due to its individualization power (van Oorschot et al., 2021). Typically, when one thinks of DNA evidence they think of blood, semen, vaginal secretions, and hair with the follicle still attached. However, many other biological substances can contain a DNA profile such as sweat, tears, vomit, feces, urine, and bone. In today's DNA workflow, the analysis of short tandem repeats (STRs) are utilized to obtain a DNA profile. STRs are when a short sequence of base pairs repeats a variable number of times at a distinct location on a chromosome. Current DNA analysis uses 20+ DNA locations to create a DNA profile. Known DNA profiles for individuals can then be compared to unknown DNA profiles left at crime scenes to ascertain whether it was a particular person's DNA left at the crime scene.

2.2 Clothing and DNA Evidence

Since its first use in forensics in the 1980s, DNA instruments have become much more sensitive (van Oorschot et al., 2021). In the past, an entire blood pool was needed to get a full DNA profile. Now, a full genetic profile can be created from a handful of cells or even a single cell (Farash et al., 2018). Instead of relying purely on large DNA samples, such as pools of blood, many everyday items are able to be sampled, such as door handles, steering wheels, personal items, and computers (van Oorschot et al., 2021). Clothing that is worn during the crime can also prove to be valuable evidence for the forensic scientist to analyze.

One study by Szkuta et al. looked at the location of DNA found on clothing worn for a full day of either work or non-work activities (Szkuta et al., 2019). The shirts were already owned by participants but were washed prior to the experiment. Some key findings came from this experiment, one of which being that the DNA from the wearer was found in all the areas

sampled, both internal and external. The sites with the most wearer DNA were the internal collars followed by the internal cuffs. Furthermore, several external sites also showed DNA of people that were in contact with the participants throughout the day. In some cases, contact that occurred early in the day was still present at the end of day sampling. There was lots of variety in the samples as to DNA quantity, profile composition, and the inclusion/exclusion of the participants and those they were in contact with previously (Szkuta et al., 2019). In another study, samples taken of the garments before the experiment showed that there was already existing DNA on the garments (Ruan et al., 2018). This shows that being laundered does not always remove all the DNA from a shirt that was worn many times before.

2.3 Laundry and DNA Evidence

Another complication that can arise in DNA evidence is criminals actively trying to destroy DNA evidence to avoid being caught. If someone thinks that their clothes have evidence on them after committing a crime, they often try to wash them free of all DNA evidence. However, with the increased sensitivity of DNA technology, this doesn't always work as intended. Both stains of commonly found biological fluids, such as blood, and skin cells from regular wear have been shown to persist on clothing even when washed with varying conditions.

There has been a lot of research into the persistence of bloodstains on fabric during laundering. Generally, studies have found that while the bloodstain itself might be lightened or even invisible after washing, there is still blood present. This has been determined through presumptive tests such as Hemastix, BlueStar (Stojanović, 2019), and luminol (Edler et al., 2017; Hofmann et al., 2019; Nakanishi et al., 2020; Ünsal Sapan et al., 2021), but also through DNA extraction and quantification (Edler et al., 2017; Hofmann et al., 2019; Salahuddin et al., 2018; Thabet et al., 2018; Ünsal Sapan et al., 2021). These studies looked at the variables that affect

how much blood is washed from clothing. Primarily, there is the difference between machine washed clothing and hand-washed clothing. Hand-washed clothing still contains bloodstains in most cases, with the exception of polyester-containing fabrics. In these fabrics, visible bloodstains are washed away (Edler et al., 2017). Another well-studied variable is water temperature. It has been found that bloodstains that are washed in higher temperature water (such as 90° C) often wash away more blood and DNA away from the fabric than lower temperature water (Edler et al., 2017; Hofmann et al., 2019; Ünsal Sapan et al., 2021). Additionally, the type of fabric is seen to be a predictor in determining how well blood is washed away from it. For instance, cotton has been seen to retain more DNA because of the absorbency of the fabric (Edler et al., 2017; Thabet et al., 2018; Ünsal Sapan et al., 2021). One study looked at only the effect of different textiles and found that amalgam fabrics, fabrics made of several types of fibers, retained more DNA than pure fabrics (Salahuddin et al., 2018). The authors also found that wool had the least amount of DNA. However, they still obtained DNA from all of the fabric samples (Salahuddin et al., 2018).

Just like biological stains, DNA derived from skin cells that transfer to clothing through regular wear are also seen to persist through the laundering process. Helmus et al. looked at several watery environments holding clothing, including a bathtub and a running tap. In these scenarios, DNA profiles could still be obtained from samples held in watery environments for the longest period that was measured in each experiment: ten minutes for the running water and one month for the bathtub experiment (Helmus et al., 2018). The bathtub scenarios even tested the use of soap and found that it didn't affect the amount or quality of the DNA profiles obtained from those scenarios. Although neither one of these scenarios is a washing machine, it has been seen that DNA from clothing can be collected and analyzed even after long periods of soaking or

rinsing. As mentioned earlier, pre-testing of owned clothing has shown that DNA of the wearer is present even if freshly washed (Ruan et al., 2018). Some of the greatest evidence for persistence of epithelial cells on clothing has been the several studies done on DNA transfer via laundry.

2.4 DNA Transfer in Laundry

Since DNA technology has become more sensitive, the transfer of small amounts of DNA from one surface or person to another has become a concern, as the DNA being found may have little or nothing to do with the crime being investigated (van Oorschot et al., 2021). This can either be through direct transfer, such as a handshake, or through indirect transfer, such as two unrelated people touching the same object, like a doorknob. The question in forensic science is no longer whether scientists will find DNA evidence, but how did the DNA get there (van Oorschot et al., 2021).

One of the scenarios of DNA transfer that has been studied is the transfer of DNA within a washing machine. In a study looking at the daily lives of everyday people, it was found that when laundry was done with the clothes of housemates, the housemates' DNA was found on the freshly laundered clothing of the participant (Szkuta et al., 2019). Studies have also been conducted looking at the transfer of DNA from worn clothing to clean clothing in a laboratory (Ruan et al., 2018; Voskoboinik et al., 2018). Both studies have seen the transfer of DNA from a load of regular worn laundry to a clean garment or patch. In one study, it was found that 76% of samples were seen to have DNA transfer (Ruan et al., 2018). In the other study, the rate of incidence was considerably lower (22%), but the phenomenon was still observed (Voskoboinik et al., 2018). This means that not only can epithelial DNA survive the laundering process, but it can also transfer onto other clothing that has never been worn before. One concern also studied is whether DNA could reside on the drum of the washing machine and transfer to clean clothes

during the wash cycle. In both studies that considered this issue, blank patches that were washed alone in the washing machine in a private washing machine showed almost no DNA (van den Berge et al., 2016) and a clean garment in a public washing machine had no detectable STR profiles (Voskoboinik et al., 2018).

Stains from biological fluids have also been shown to transfer in the washing machine. Most research has focused on semen stains transferring in the washing machine along with a couple of studies on vaginal secretions. A study conducted in 2016 found that semen and vaginal secretions could be found on pristine clothing after laundering them together, which could have severe implications for children's sexual abuse cases (Noël et al., 2016). In many children sexual abuse cases, laundry is used as evidence, but this study showed that the family washing laundry together could lead to DNA on children's items even when no abuse has occurred.

Even though blood is one of the most commonly found biological fluids found at a crime scene, fewer studies have been conducted about the transfer of blood stains in washing machines. Kamphausen et al. found that full or partial genetic profiles were found in many of the clean patches that were washed with a patch with blood on it (Kamphausen et al., 2015). In another study, the authors weren't trying to study DNA transfer in blood but rather persistence of blood stains on clothing during laundering (Hofmann et al., 2019). However, they discovered that in their first study round, there were no differences in the treatments because they had all been washed together and transferred blood between them. This was supported by the fact that the control pristine patches included showed similar levels of luminescence using a presumptive test. Later, it was shown in the same study that the fullness of the laundry machine had an effect on DNA transfer. Washing machines that were more heavily filled prevented the original stain from being washed, but also diluted the blood released and therefore the DNA transferred to pristine

cloths (Hofmann et al., 2019). DNA transfer in the washing machine is a problem that has been observed in research and could pose a problem for interpretation of DNA evidence, especially in the case of stains where more DNA is present.

2.5 Laundry Sanitizers

In the United States today, most households use cooler temperature washes and less laundry additives such as bleach (Abney et al., 2021; Bockmühl, 2017; Bockmühl et al., 2019). However, this means that not all the microbes are being washed from clothing. It has been seen that pathogenic microbes can exist on clothing even after going through the washing machine (Whitehead et al., 2022). This is where other laundry products can be used to have an antimicrobial effect. Laundry sanitizers are laundry products that became much more common in their use in the United States since the pandemic. Laundry sanitizers are products whose purpose is to kill bacteria and viruses more effectively than regular laundry detergent. The active ingredients in laundry sanitizers are commonly quaternary ammonium (QAC) compounds, specifically benzalkonium chloride (BAC) and dimethyl dodecyl ammonium chloride (DDAC) (Bockmühl, 2017; Bockmühl et al., 2019). Quaternary ammonium compounds are cationic in nature, allowing them to interact with negatively charged textiles. Since the QACs are cationic and typical laundry detergents are anionic, they cannot be utilized at the same time. Therefore, QACs are either applied during an extra presoak step or applied as a rinse after the detergent is used. It is presumed they stay on the clothing fibers even after the wash is over, providing some lasting antimicrobial effect (Bockmühl, 2017; Bockmühl et al., 2019). This ionic ability of QACs is used to disrupt the cell membrane of bacteria and viruses.

When laundry sanitizers are considered, it is mostly testing their effectiveness of removing microbes, since this is their intended purpose. One study inoculated cloths with the

viruses being studied and then suspended these cloths in the laundry sanitizers (Khalid Ijaz et al., 2022). The QAC-based sanitizers caused a large inactivation of SARS-CoV-2. It also tested the effectiveness of the sanitizers of removing viruses from hard surfaces as well to simulate the sanitizer removing viruses from the laundry machine itself. In this case, the QAC-based sanitizer led to a complete inactivation of all the coronaviruses and influenza viruses tested (Khalid Ijaz et al., 2022). Although this wasn't a study that utilized an actual washing machine and used a washing cycle, it is a good starting point to realize that QAC-based laundry sanitizers perform as they are designed for. Another study looked at the effectiveness of laundry sanitizers on removing one specific pathogenic bacteria from the turnout gear of firefighters (Farcas et al., 2019). The study found that the laundry sanitizer was typically very effective at removing the pathogen from the turnout gear. However, it didn't perform well on turnout gear that was more water resistant. The authors hypothesized that the laundry sanitizer couldn't fully penetrate the turnout gear and inactivate the bacteria that were deeply embedded in the material. The average piece of clothing isn't water resistant, so laundry sanitizers should be quite effective on regular clothing, as shown by the lack of pathogen on the turnout gear that wasn't water resistant (Farcas et al., 2019).

As of right now, not much research has been done to see whether laundry sanitizers affect the amount of DNA removed during the laundry process. Typically, when laundry products other than detergent are studied, the laundry products used are stain removers (Edler et al., 2017; Hofmann et al., 2019; Houston, 2016; Thabet et al., 2018), fabric softeners (Houston, 2016), and active oxygen compounds (Houston, 2016). Results have been quite varied in the success of these products on removing DNA. In one study, stain remover alone removed DNA better than a regular detergent (Thabet et al., 2018). However, in another study, it was found that stain

remover by itself wasn't very effective at removing blood stains from cloth, and the blood stains had high visibility after washing (Edler et al., 2017). This same study included a product known as a hygiene rinse, which seems to be similar to a laundry sanitizer. In this study, the hygiene rinse also didn't do well at removing the bloodstain, leaving the blood visible on the cloth (Edler et al., 2017). Although this particular study didn't quantify the amount of DNA that was present in the blood stains after washing, it still looked at the usefulness of laundry additives on removing biological evidence from clothing.

2.6 Collection Methods

Currently, there are several different methods for collecting DNA evidence from items found at the crime scene. They include dry swabbing, wet swabbing, adhesive tape lifts, cutting the object, and scraping if there is a stain present. Copious amounts of research have shown that the best collection method is dependent on the substrate that is being sampled (Blackmore et al., 2024; Comte et al., 2019; Hedman et al., 2021; Hymus et al., 2024; McLamb et al., 2020; Sessa et al., 2019; Vickar et al., 2018; Währer et al., 2023). When researched, swabs often collect higher amounts of DNA on nonporous substrates than other collection methods (Sessa et al., 2019). On the other hand, swabs often perform badly when sampling porous substrates, and methods such as adhesive tapes and cuttings work better. Even when comparing swabs made of different materials or different sizes, different materials will perform better for some substrates than others (Hedman et al., 2021). In one study, swabs were found to perform the same on nonporous surfaces, but large foam swabs performed better on wood surfaces and cotton swabs performed better on glass (Hedman et al., 2021). In another study, it was found that nylon flocked swabs performed better on cotton shirts and steering wheels than cotton swabs, but not screwdrivers (Comte et al., 2019). The same surface dependent efficiency of collection has been seen in adhesive tapes as well. One study found that fabrics more likely to have PCR inhibitors,

such as denim, were better processed using a tape with less adhesiveness, since it collected a comparable amount of DNA without also collecting PCR inhibitors (Hymus et al., 2024).

Porous and rough substrates can be problematic when it comes to collecting evidence. The roughness of the substrate makes it difficult for tapes and swabs to collect all the DNA that may be in crevices. A new method has been developed to try and combat this issue. The method is known as the Microbial Vacuum Wet-Vacuum System (M-Vac™) and its goal is to increase DNA collection on porous and large surfaces. It was not originally designed for this purpose but was instead designed for the food safety industry in order to detect pathogens on surfaces (*History, n.d.*). However, the forensic science field realized that it could potentially be used for DNA collection and has since adopted it for use on porous and rough surfaces after the components were made single use.

The M-Vac™ system works by spraying sterile buffer onto the item of evidence in order to loosen the DNA embedded inside (Blackmore et al., 2024; McLamb et al., 2020; Vickar et al., 2018). It then simultaneously vacuums up the buffer for collection into a vessel (Figure 1). The collected liquid is then put onto a polyethersulfone (PES) filter which allows the buffer to pass through and leaves the DNA on the filter. However, it is important to note that the filter only captures cells, any cell free DNA passes through the filter and is lost (Vickar et al., 2018). This is one potential downside to the M-Vac™, as the cell free component can contain crucial information. Once collected, the filter itself can then be processed in a DNA workflow.

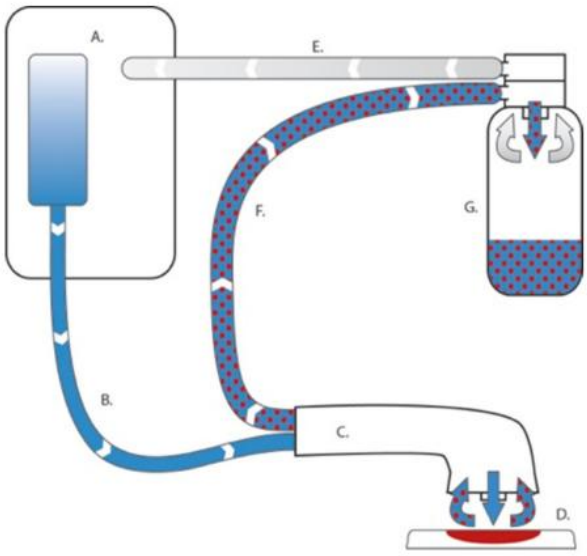

Figure 1. Diagram describing how the M-Vac™ works to collect DNA. Adapted from (Garrett et al., 2014).

When the M-Vac™ is compared to other conventional collection methods, its usefulness is once again dependent on the type of surface being sampled (Blackmore et al., 2024; McLamb et al., 2020; Vickar et al., 2018; Währer et al., 2023). When nonporous surfaces were researched, it was found that the M-Vac™ performed equal to (McLamb et al., 2020) or worse than (Vickar et al., 2018; Währer et al., 2023) the conventional swabbing method. On tile, it was seen in one study that conventional swabbing performed better than the M-Vac™ (Vickar et al., 2018), but another study found that the M-Vac™ performed equally well to conventional methods like taping and swabs (Garrett et al., 2014). This lack of utility on nonporous substrates may be due to the fact that research has shown that the M-Vac™ can propel DNA away from the item of evidence, up to four inches away from where the DNA was distributed on the evidence (Garrett et al., 2014). From this, it is clear that the M-Vac™ should not be used on nonporous substrates, as it at best only performs comparably to the conventional methods.

However, when porous surfaces were investigated, the M-Vac™ performed better. The surfaces studied so far are varied but include sweaters, construction materials such as bricks, automotive items, and household items such as drywall, stone, and wood (Blackmore et al., 2024; McLamb et al., 2020; Vickar et al., 2018; Währer et al., 2023). When 22 different substrates were spotted with diluted bloodstains, it was seen that the M-Vac™ performed better on 18 of the substrates by sometimes as much as 12 times greater quantities. Twenty of the substrates were porous and two were nonporous; only two porous substrates and the two nonporous substrates performed similarly when compared to moistened swabs (McLamb et al., 2020). Some contradictory findings exist for carpets, with one study finding that conventional swabs and tapes worked better with touch DNA while another study found that the M-Vac™ collected more DNA on saliva stains (Blackmore et al., 2024; Währer et al., 2023).

In mock case scenarios, the M-Vac™ system was more helpful when the evidence items were soiled or there was only one contributor expected (Blackmore et al., 2024). Since the M-Vac™ is made for large surfaces, it can often collect background DNA that was not from the contributor profile, causing complex DNA mixtures for the DNA analyst to interpret (Blackmore et al., 2024). Another very important aspect of the M-Vac is that it can be used on evidence after conventional collection techniques have already been used. In three different substrates, the M-Vac collection was significantly larger than the initial swabbing and had an average of 10 times more DNA using the M-Vac versus the initial swabbing (McLamb et al., 2020). The M-Vac™ system is a new technique that has many advantages and shows great promise in the field already by being used in cold cases, but the M-Vac™ needs more research before it can be used to its full potential.

2.7 Significance of Research

DNA transfer and persistence have become topics that have been heavily researched in the forensic field in the last several years (van Oorschot et al., 2021). Specifically, research into the persistence and transfer of DNA during the washing process has received a lot of attention from researchers. It has been shown time and time again that at least some DNA survives washing protocols in many cases. Some variables affect persistence and transfer, such as water temperature, hand-washing vs machine-washing, and added laundry products (Edler et al., 2017; Hofmann et al., 2019). The area that has received the least amount of research for how diverse the options are laundry products. With the other variables (water temperatures, hand-washing vs machine-washing), the options are few or even between two options. The options of laundry products are wide and varied.

Laundry products come in many different forms. There are conventional laundry detergents that are added to every load of laundry, but there are other products that not every person uses or gets used only in special cases. These products are more likely to be stain removers, bleach products, scent boosters, etc. (Edler et al., 2017; Houston, 2016; Thabet et al., 2018). A laundry product of particular interest is laundry sanitizer. Laundry sanitizers are an added product that aim to kill more bacteria and viruses than a conventional laundry detergent. They have become increasingly popular with the public, and this is shown by all of the different brands that have released laundry sanitizers in recent years. However, these laundry sanitizers have received very little attention in literature. Other laundry products of interest are commercial laundry products. There are several laundry products utilized by dry cleaners or professional laundry services that are not typically available to the public directly. These laundry products come in all sorts of formulations, and there has been very little research on any commercial laundry products. This

thesis project aims to investigate both laundry sanitizers, sampling from a variety of brands, and some select commercial laundry products.

Collection of DNA has been another area that has gotten more attention from researchers in order to collect more quantity and quality information. One specific area of interest has been sampling from porous substrates, which is often difficult to accomplish. A potential solution is the use of wet vacuuming using the M-Vac™ (Blackmore et al., 2024; Vickar et al., 2018). However, the M-Vac™ needs a lot more research done to understand when it is best utilized. Many substrates have already been researched, including clothing, but laundered clothing especially has not been seen in the literature. This thesis project aims to add to the collective knowledge of the usefulness of the M-Vac™ in collecting DNA evidence from porous objects.

3. Methods

3.1 Managing Background DNA

The washing machine used to wash all the samples was a private washing machine of one of the researchers. Therefore, extra concern was taken to make sure that background DNA from the washing machine doesn't affect the study. A Tide™ washing machine cleaner pouch was used in conjunction with the sanitizing cycle at the start of the study, in between samples, and at the end of the study. After each sanitizing cycle, the drum of the washing machine was physically wiped down using Clorox™ disinfecting wipes. In addition, a control cotton swab was used on the drum of the washing machine. This control swab was processed via DNA extraction and quantitation to determine if DNA was present after this cleaning process.

3.2 Specific Aim 1: Evaluation of DNA Persistence in a Washing Machine

3.2.1 Preparation of Samples

A male participant, for whom informed consent was obtained according to the University of Central Oklahoma's Institutional Review Board (IRB), put on a brand-new 100% cotton Fruit of the Loom T-shirt. The cotton T-shirt, although brand new, was still treated for any potential background DNA. This was done by placing the T-shirt in the Spectrolinker, an XL-1500 UV crosslinker from Spectronics Corporation, to be exposed to ultraviolet (UV) light for fifteen minutes. This was repeated for both the inside and the outside of the shirt, taking extra care to expose the underarm area and collar as these were the sampling areas. After putting on the shirt, the participant engaged in thirty minutes of exercise, having been instructed to not wear any deodorant that may prevent DNA transfer to the T-shirt. After the thirty minutes of exercise had concluded, the T-shirt was collected from the participant.

After DNA was deposited on the T-shirt, the shirt was prepped for washing. The T-shirt was cut in half vertically to create two identical halves. Since the areas of collection were the underarm region and collar, excess fabric in the midsection region was cut away to prevent excess DNA transfer. One T-shirt half was pre-soaked in the laundry product treatment before being washed in the washing machine, an LG Inverter Direct Drive. The other half of the T-shirt had the laundry product treatment included in the rinse cycle of the washing cycle. This step was performed in consideration that many of the laundry sanitizers have two sets of directions: one for pre-soaking and one for adding to the rinse cycle. There were six tested laundry products with two added laundry products acting as controls. The six laundry products being tested were Lysol™ Laundry Sanitizer, OxiClean™ Laundry & Home Sanitizer, Tide™ Hygienic Clean Heavy Duty 10X Liquid Detergent, Clorox™ Laundry Sanitizer Liquid, Silver Satin 2+ Bleach, and Faultless

Star Power. For a positive control, Clorox™ Disinfecting Bleach was used as bleach is commonly used in forensic laboratories to eliminate DNA. For a negative control, conventional laundry detergent, specifically Tide™ Plus Sport Odor Defense, was used to demonstrate the average washing product of a household. All of the treatments have been expanded on in Table 1. The same washing cycle was used for each of the T-shirt halves, with the same duration and water temperature. After the T-shirt halves were finished with the washing cycle, they were set to air dry to avoid further variables associated with the dryer. Once dry, they were packaged and transported to the lab for DNA collection.

Table 1: Laundry products utilized in the study with their main active ingredients and instructions.

Product	**Main Active Ingredients**	**Instructions**
Clorox™ Disinfecting Bleach (Clorox, 2024)	Sodium hypochlorite	Fill bleach to max line dispenser or add measured amount of bleach to wash water. Add ¼ cup for HE machines. Add clothes and start wash. Ensure contact with bleach for 10 minutes.
Clorox™ Laundry Sanitizer Liquid: Clean Linen (Clorox, 2025)	Alkyl C12-16 Dimethyl benzyl Ammonium Chloride, octyl decyl dimethyl ammonium chloride, dodecyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride	Sanitize: Add to rinse cycle and wash as usual. Disinfect: Pour 4 capfuls into 1 gallon of water. Submerge and soak for 15 minutes. Rinse thoroughly with clean water or machine wash as directed.
Faultless Star Power (Faultless, 2021)	Disodium carbonate, sodium percarbonate, sodium dodecylbenzenesulfonate	Used Tide cap to measure. Used powder up to line 5 (approx. ½ cup)
Lysol™ Laundry Sanitizer Free and Clear (Lysol®, 2023)	Alkyl dimethyl benzyl ammonium chloride, octyl decyl dimethyl ammonium chloride, dodecyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride	Sanitize: Add 2 capfuls each filled to line 2 to rinse cycle and leave product for 16 minutes. Disinfect: Pour 2 capfuls into 1 gallon of cold water. Soak for 15 minutes. Rinse thoroughly with

		clean water or machine wash as directed.
Oxiclean™ Laundry & Home Sanitizer (OxiClean, 2020)	Disodium carbonate, sodium percarbonate, alcohols C12-C15 ethoxylated, tetraacetylethylenediamine	Sanitize: Fill scoop to the top once before adding laundry. Add detergent. Wash 15 minutes in warm water. Disinfect: Fill the scoop to the top once and add to one gallon of water. Immerse laundry for 15 minutes for bacteria and viruses, or 30 minutes for fungi prior to starting the wash cycle.
Silver Satin 2+ Bleach (Faultless, 2024)	Sodium percarbonate	Used Tide cap to measure. Used powder up to line 5 (approx. ½ cup)
Tide™ Hygienic Clean Heavy Duty 10X Liquid Detergent Original Scent (Tide, 2024)	C10-16 pareth, Sodium and MEA C10-16 Alkylbenzenesulfonate, Sodium lauryl sulfate, C14-15 Parenth-n, MEA laureth sulfate	Add detergent, then add clothes. Medium loads fill to bar 1 on cap.
Tide™ Plus Febreze Sport Odor Defense Liquid Laundry Detergent (Tide, 2020)	Sodium Laureth Sulfate, Sodium C10-16 Alkylbenzene Sulfonate, Sodium Lauryl Sulfate, Mea- dodeculbenzenesulfonate	Measure with cap. For Medium Loads, fill to bar 1 on cap. Add detergent, then add clothes.

3.2.2 DNA Collection

Once ready for DNA collection, the T-shirt half was unpackaged and placed on a sterile workplace. A sterile scalpel was used to cut out the underarm area of the shirt, as this area would be expected to retain the most DNA due to sweat. This underarm area of the shirt was then further cut in half. Each half was collected with one of two methods: cotton swab or M-Vac™.

For the cotton swab method, two sterile cotton swabs were unwrapped and moistened with deionized water. After being moistened, the two swabs were put to the shirt section and firmly rubbed across the surface of the T-shirt section. Once the whole section was swabbed, the swabs were cut using a sterile scalpel and placed into separate, labeled 1.5 ml centrifuge tubes.

This created analytical triplicates for each tested scenario. The tubes were stored in a -20° C freezer for storage until further processing.

For the M-Vac™ method, the shirt section was placed in a plastic weigh boat to catch any potential buffer that soaked through the shirt section. Once the shirt section was in the weigh boat, the buffer was applied and the M-Vac™ head used to vacuum the buffer simultaneously. The M-Vac™ head was applied to the shirt section in vertical passes, making sure to overlap passes to ensure complete DNA collection. Pressure was applied during the passes to press more buffer out of the shirt section. The DNA filtrate was then poured through the filter under vacuum pressure, focusing on the center of the filter. The filtrate was poured through the filter twice to ensure the most DNA possible was captured on the filter. The filter was then allowed to air dry. The filter was then cut out and cut into thirds using a sterile scalpel. This created analytical triplicates for each tested scenario. Each filter third was cut into small pieces (approximately 0.25 cm – 0.5 cm squares) with a sterile scalpel in order to maximize surface area available for the reagents in the DNA workflow. The pieces were then carefully placed into separate, labeled 1.5 ml centrifuge tubes. The tubes were stored in a -20° C freezer for storage until ready for further processing. The sampling process utilized for testing is illustrated in Figure 2.

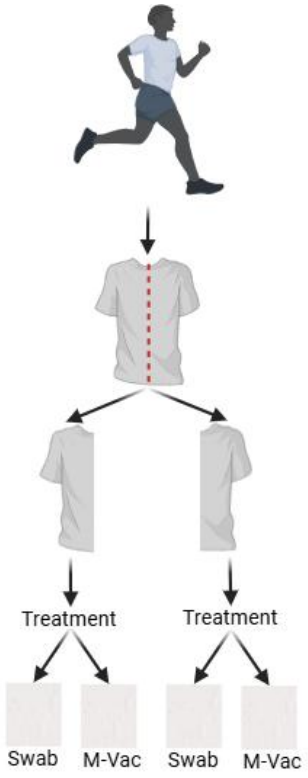

Figure 2. A visual representation of the methods utilized in 3.2 Specific Aim 1

3.2.3 Bacteria Colony Assessment

Bacteria colony collection was performed at the same time as the DNA collection. A sterile swab was moistened with sterile water and applied to one third of the collar of the T-shirt. The swab was then gently streaked back and forth on one third of a labeled LB agar plate from EZBioResearch. This was repeated with separate swabs twice more for a total of three replicates for each shirt, with each replicate being on the same agar plate. The agar plates were then allowed to incubate at room temperature in a dark drawer for 96 hours. After the incubation period, the plates were inspected for bacterial growth. Colonies were counted for each third of the plate and any additional observations were noted.

3.3 Specific Aim 2: Evaluation of DNA Transfer in a Washing Machine

3.3.1 Preparation of Samples

Washcloths (100% cotton, 12 inches by 12 inches) from Utopia Towels were obtained and treated to prevent any potential background DNA from influencing the data. This was done by placing each washcloth in the Spectrolinker, an XL-1500 UV crosslinker from Spectronics Corporation, and exposing the washcloth to UV light for fifteen minutes. This was done for both sides of the washcloth. Once treated, whole human blood from a male was applied to the washcloths using a micropipette. The blood was purchased from the Oklahoma Blood Institute. One of two amounts of blood was applied to each washcloth, either 25 µL labeled "1 drop" or 75 µL labeled "3 drops". The blood was allowed to air dry. The labels were also cut off to distinguish between washcloths that were not spotted with blood. Once the blood was dry, the washcloths were packaged prior to washing.

For the washing step, a bloodstained washcloth and a clean washcloth were placed in an LG Inverter Direct Drive washing machine. Four laundry products were evaluated, along with two additional products designed as controls. The four laundry products being tested are Lysol™ Laundry Sanitizer, OxiClean™ Laundry & Home Sanitizer, Tide™ Hygienic Clean Heavy Duty 10X Liquid Detergent, and Clorox™ Laundry Sanitizer Liquid. Clorox™ Disinfecting Bleach was used for a positive control, as it is commonly employed in forensic laboratories for DNA degradation. For a negative control, conventional laundry detergent, specifically Tide™ Plus Sport Odor Defense, was used to demonstrate the average washing product of a household. Details about the laundry products can be found in Table 1. The same washing cycle was utilized for each washcloth pair, with the same duration and water temperature. After the washing cycle

was complete, the initially clean washcloth was collected and allowed to air dry. Once dried, the washcloth was packaged and stored at room temperature until DNA collection.

3.3.2 DNA Collection

When ready for DNA collection, the washcloth was unpackaged and DNA was collected via one of two methods: the swabbing method or the M-Vac™ method. One method was applied to one half of the washcloth, and the other method was applied to the other half of the washcloth.

For the cotton swab method, two sterile cotton swabs were unwrapped and moistened with deionized water. After being moistened, the two swabs were firmly rubbed across the surface of the washcloth. Once the whole section was swabbed, the swabs were cut using a sterile scalpel and placed into separate, labeled centrifuge tubes. This created analytical triplicates for each tested scenario. The tubes were stored in a -20° C freezer for storage until further processing.

For the M-Vac™ method, the washcloth was placed on a baking tray that had been decontaminated with bleach. A tray was utilized during the collection process to catch any potential buffer that soaked through the washcloth. Once the washcloth section was on the baking tray, the buffer was applied and the M-Vac™ head used to vacuum the buffer simultaneously. The M-Vac™ head was applied in vertical passes, making sure to overlap with the previous pass to ensure complete collection. Pressure was applied during the passes to press more of the buffer out of the washcloth. The DNA filtrate was then poured through the filter while under vacuum pressure, focusing on the center of the filter. The filtrate was poured through the filter twice to ensure the most DNA possible was captured on the filter. The filter was then allowed to air dry. The filter was then cut out and cut into thirds using a sterile scalpel. This

created analytical triplicates for each tested scenario. Each filter third was cut into small pieces (approximately 0.25 cm - 0.5 cm squares) with a sterile scalpel in order to maximize to surface area available for the reagents in the DNA workflow. The pieces were then carefully placed into separate, labeled 1.5 ml centrifuge tubes. The tubes were stored in a -20° C freezer for storage until ready for further processing (Figure 3).

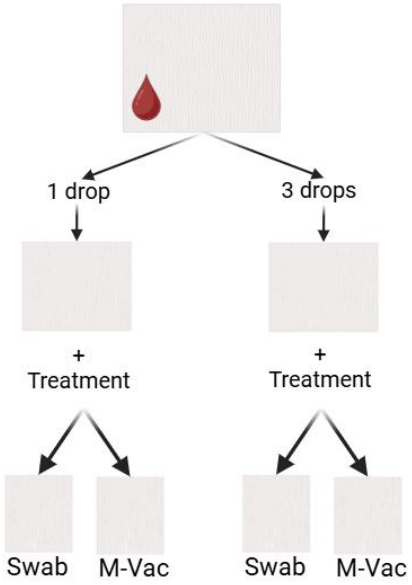

Figure 3. A visual representation of the methods utilized in 3.3 Specific Aim 2.

3.4 DNA Extraction

All of the samples were extracted using the Promega DNA IQ™ extraction kit. The samples were processed using the manufacturer's protocol, found in *DNA IQ™ System – Database Protocol,* specifically *DNA Isolation from Stains and Buccal Swabs* (Promega, 2016). 250 µL of lysis buffer containing 1M DTT was added to each sample in a microcentrifuge tube. The samples were then incubated at 70° C for 30 minutes. After incubation, the samples were transferred to a spin basket and centrifuged at maximum speed for two minutes. The spin basket was then discarded. The bottle of resin was vortexed and 7 µL of resin added to each sample. The

samples were incubated at room temperature for three minutes, vortexing the sample every minute. The samples were then put on a magnetic stand. The solution was carefully discarded without disturbing the pellet. 100 µL of lysis buffer, containing 1M of DTT, was added to the tube. The samples were vortexed and then immediately put back on the magnetic stand. Again, the lysis buffer was carefully removed without disturbing the resin pellet. 100 µL of wash buffer was added to the tubes and vortexed before placing back on the magnetic stand. The wash buffer was carefully removed without disturbing the pellet. This wash buffer step was repeated once more. After the washing steps, the tops to the tubes were left open and the tubes allowed to air dry for five minutes while on the magnetic stand. 25 µL of elution buffer was added to the tubes, vortexed, and then left to incubate at 65° C for five minutes. After incubation, the tubes were vortexed and placed back on the magnetic stand for a final time. The resulting elution buffer was carefully transferred to a clean microcentrifuge tube. The final tubes were stored in a -20° C freezer until quantitation.

3.5 DNA Quantitation

All of the samples were quantitated with the same kit, namely the Quantifiler™ Trio DNA Quantitation Kit. The samples were processed using the manufacturer's protocol found in *Quantifiler™ HP and Trio DNA Quantification Kits User Guide* (AppliedBiosystems, 2018). Reagents were prepared by thawing and briefly vortexing the Quantifiler™ Primer Mix before centrifuging, and briefly vortexing the Quantifiler™ PCR Reaction Mix. A master mix of the reagent was created by adding 10 µL of PCR reaction mix and 8 µL of primer mix, respectively, per sample to a microcentrifuge tube. The master mix was vortexed briefly to ensure the master mix was properly incorporated. Then, 18 µL of master mix was added to each of the wells of the plate. 2 µL of sample, standard, or control was added to the correct wells of the plate. The plate

was sealed with the Optical Adhesive Cover and each well of the plate firmly sealed off by applying pressure. The plate was gently tapped against the countertop to break up any bubbles that may have gotten trapped. The plate was then briefly centrifuged for a couple of seconds to remove the bubbles from the wells that would interfere with the reaction. After being centrifuged, the plate was put on an Applied Biosystems QuantStudio™ 5. The standard and sample names were entered into the software using the plate set-up and ran. The samples were initially heated for two minutes at 95° C. They were then subjected to forty rounds of PCR, which consisted of nine seconds of 95° C heat followed by thirty seconds of 60° C.

3.6 DNA Amplification

A select few samples were chosen to be amplified with the end goal of assessing the electropherograms. The eleven samples with the highest DNA concentrations were chosen along with a couple of low concentration samples for comparison. All of these chosen samples were amplified using the PowerPlex® Fusion 5C System kit. The manufacturer's protocol was followed, found in the *PowerPlex® Fusion System for Use on the Applied Biosystems Genetic Analyzers Technical Manual* (Promega, 2020). First, a master mix was created using the PowerPlex® Fusion 5X Master Mix and the PowerPlex® Fusion 5X Primer Pair Mix. 2.5 µL of each component were added per sample to a microcentrifuge tube. The master mix was vortexed to properly mix and 5 µL of the master mix added to each reaction well. Then, 7.5 µL of the sample was added to each reaction. For the positive control, 7.5 µL of 2800 M Control DNA was diluted to 0.5 ng/µL using amplification grade water and was added to a tube containing the 5 µL amplification master mix. For the negative control, 7.5 µL of amplification grade water was added to a tube containing the 5 µL of master mix. The tubes were tapped against the counter to remove any potential bubbles. The tubes were then placed onto an Applied Biosystems

GeneAmp® PCR System 9700. The tubes were initially heated at 96° C for one minute. Then, the tubes underwent thirty rounds of PCR, which consisted of ten seconds at 94° C, one minute at 59° C, and thirty seconds at 72° C. Finally, the tubes were heated at 60° C for ten minutes and left on hold at 4° C until removed from the machine.

3.7 Genetic Analysis

The select amplified DNA samples were separated and detected via capillary electrophoresis on an Applied Biosystems™ 3500 Series Genetic Analyzer. The preparation for capillary electrophoresis followed the manufacturer's protocols as found in *PowerPlex® Fusion System for Use on the Applied Biosystems Genetic Analyzers Technical Manual* (Promega, 2020). A master mix was created by adding 0.5 µL of internal land standard (WEN) with 9.5 µL of formamide per amplified sample. The master mix was then vortexed to properly mix. Then, 10 µL of the master mix was pipetted into each well. Finally, 1 µL of the amplified sample or Allelic Ladder Mix was added to each well. An allelic ladder was run for each injection column. The plate was sealed with the septa and the plate briefly centrifuged at 3,000 rpm to remove any air bubbles from the wells. The plate was then denatured at 95° C for three minutes and immediately chilled on crushed ice following denaturation. The plate was placed in a plate base, covered with a plate retainer, and loaded into the pre-calibrated genetic analyzer. The run was then conducted.

3.8 Data Analysis

The electropherograms obtained through capillary electrophoresis were analyzed using OSIRIS 2.16 (*OSIRIS*). The electropherograms were assessed for quality through the presence of artifacts and the completeness of alleles present at each locus. The concentrations of DNA for the samples were compared to one another to determine if the type of sanitizer, three drops versus one drop, soak versus no soak, or collection method had an effect on DNA concentration after

laundering. The DNA concentrations are based on three different targets: small autosomal, large autosomal, and Y concentrations. The small autosomal targets are found in the chromosomes that are not biological sex specific and have a small length in terms of base pairs, typically 75-80 bp (AppliedBiosystems, 2018). These smaller targets are less prone to degradation because of their size. Meanwhile large autosomal targets are from chromosomes that are not biological sex specific and are larger in size, usually greater than 200 base pairs. These targets are more prone to degradation but are helpful in assessing whether degradation has occurred. Finally, Y targets are found on the Y chromosome and are therefore specific for biological male DNA.

Data analysis was completed using R v. 4.5.2. using RStudio 2026.01.0+392 (*R: A language and environment for statistical computing*, 2018). A hurdle model was used as it helps to account for data where there are a large number of zeros present that prevent other statistical models from being used. Essentially, the hurdle splits each model into two: a logistic regression that models the probability of complete elimination and a linear regression on the log-transformed data to model the non-zero values. For the elimination model, penalized profile likelihood confidence intervals were calculated. For the non-zero model, Wald confidence intervals and geometric mean ratios (GMR) were calculated for the log-transformed data. P-values were obtained for each variable being considered: type of laundry sanitizer, no soak versus soak, three drops versus one drop, and collection method. Interactions of the effects were not considered due to limited replication. The brands of laundry sanitizer were grouped together by ingredients in order to allow for ease of data analysis. The QAC group contains Lysol™ and Clorox™, the Miscellaneous group contains OxiClean™ and Tide™ 10X, the Dry Cleaning group contains Faultless Star Power and Silver Satin 2+, and the controls (Clorox™ Bleach and Tide™ Sport) remain separate from any group.

4. Results

4.1 Specific Aim 1: Evaluation of DNA Persistence in a Washing Machine

The results for the DNA concentrations of the samples from Specific Aim 1 are listed below in Table 2. There is the average DNA concentration, standard deviation, and the number of samples with no detectable DNA with respect to the variables of detergent group, method, and soak versus no soak. There were a large number of zeros in the data, approximately 15% of samples for both large autosomal and small autosomal and 71% of samples for Y DNA. The presence of these zeros influenced the type of statistical analysis that could be performed on the data. Even when there was quantitative concentration data, the concentrations were very low.

Table 2. Descriptive statistics for raw DNA concentrations for Specific Aim 1.

		Large DNA			Small DNA			Y DNA		
	n	mean	s.d.	zeros†	mean	s.d.	zeros†	mean	s.d.	zeros†
Detergent Group										
low control	6	0.00070	0.00073	1	0.00067	0.00074	2	0.00020	0.00040	4
dry cleaning	12	0.00072	0.00080	2	0.01748	0.05758	1	0.00003	0.00008	10
QAC	12	0.00112	0.00131	2	0.00139	0.00137	2	0.00033	0.00042	6
miscellaneous	12	0.00396	0.00874	2	0.00701	0.01268	2	0.00001	0.00005	11
high control	6	0.00296	0.00368	0	0.00468	0.00679	0	0.00045	0.00060	3
Method										
control	16	0.00383	0.00776	3	0.00529	0.00964	1	0.00028	0.00047	11
swab	16	0.00128	0.00125	2	0.00302	0.00722	3	0.00012	0.00036	14
M-vac	16	0.00061	0.00069	2	0.01310	0.04993	3	0.00012	0.00017	9
Soak										
no soak	24	0.00243	0.00619	3	0.01271	0.04109	3	0.00022	0.00041	16
soak	24	0.00139	0.00240	4	0.00156	0.00251	4	0.00013	0.00029	18

† Number of samples with no detectable DNA

4.1.1 Large Autosomal Data

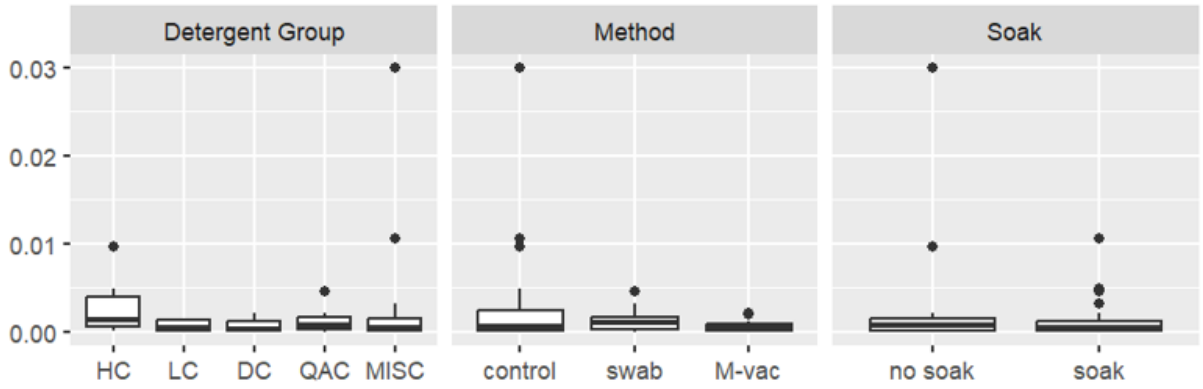

Figure 4. Boxplots of large autosomal DNA concentration from Specific Aim 1.

Although the large autosomal had less zeros than the Y DNA concentrations, the concentrations were still very low across the various variables studied. This can be seen in the boxplots showing the large autosomal DNA concentrations for Specific Aim 1 (Figure 4). Nonetheless, statistical analysis was still conducted.

The large autosomal data was split into two models as described earlier in 3.8 Data Analysis. When the elimination model was considered, none of the variables had p-values that were significant, ranging from 0.445 – 0.916 (Table 3). The confidence levels are also wide, showing the lack of precision. However, in the non-zero model, there was a variable that was significant with a p-value of 0.047 (Table 3). This geometric mean ratio means that the collection method influenced the non-zero DNA concentrations. The M-Vac™ was associated with 65% lower DNA concentrations compared to the control swabs collected from the drum of the washing machine (Figure 5). The other variables, detergent group and soak condition, were not found to be statistically significant in the non-zero DNA concentrations.

Table 3. Hurdle model with two-part regression analysis of large autosomal DNA elimination and residual concentration for Specific Aim 1.

Comparison	Elimination† OR (95% CI)	*p*-value	Residual Concentration‡ GMR (95% CI)	*p*-value
Detergent Group				
dry cleaning vs. low control	0.89 (0.10-11.0)	0.916	1.23 (0.28, 5.37)	0.775
QAC vs. low control	0.89 (0.10-11.0)	0.916	2.15 (0.50-9.35)	0.296
miscellaneous vs. low control	0.89 (0.10-11.0)	0.916	2.41 (0.56-10.5)	0.231
high control vs. low control	0.30 (0.002-6.41)	0.445	3.04 (0.61-15.3)	0.170
Method				
swab vs. control	0.69 (0.11-3.83)	0.666	0.89 (0.32-2.50)	0.821
M-vac vs. control	0.69 (0.11-3.83)	0.666	0.35 (0.12-0.99)	0.047
Soak				
soak vs. no soak	1.31 (0.31-6.00)	0.715	0.84 (0.36-1.94)	0.676

† Elimination Model: Odds ratios (OR) derived from a penalized (Firth) logistic regression, modeling the probability of complete large DNA elimination. (penalized likelihood ratio test: $p = 0.992$)

‡ Residual Concentration Model: Geometric mean ratios (GMR) obtained by exponentiating coefficients from a linear regression model, fit to log-transformed positive large DNA measurements. (global F-test: $F(7, 33) = 1.30$, $p = 0.281$)

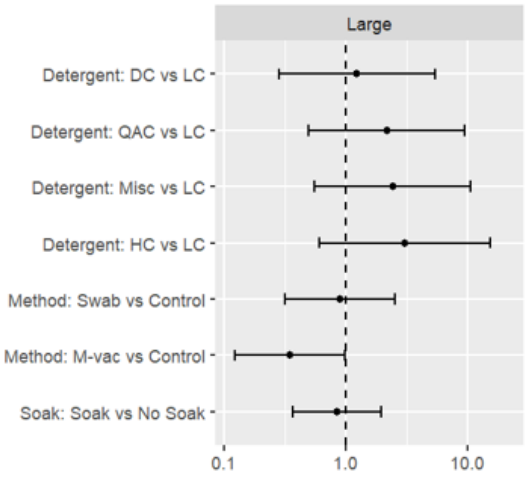

Figure 5. Adjusted geometric mean ratios (log scale) for large autosomal residual DNA concentration for Specific Aim 1.

4.1.2 Small Autosomal Data

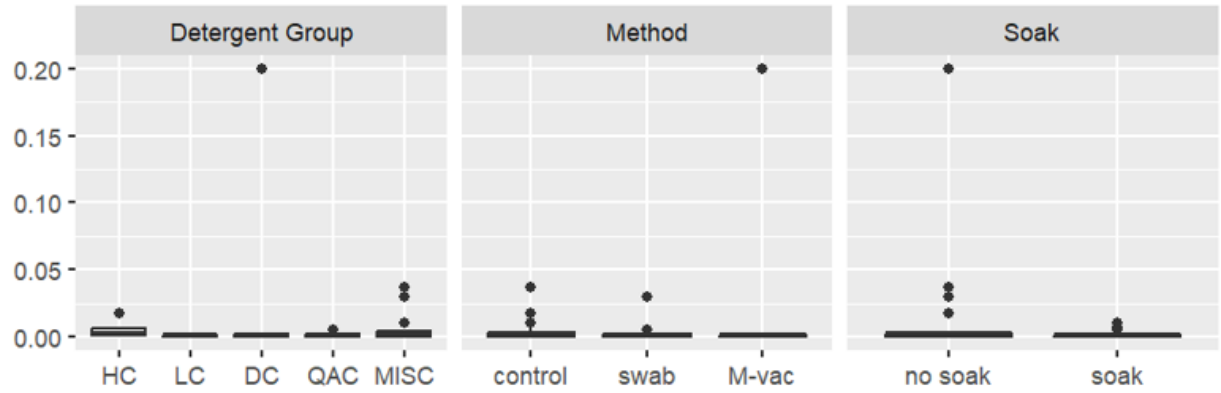

Figure 6. Boxplot of small autosomal DNA concentrations for Specific Aim 1

Although the small autosomal DNA concentrations had more non-zero data than the Y DNA concentrations, the concentrations are very low as indicated by the boxplots of the concentration data for the studied variables (Figure 6). Data analysis was still conducted to see if there were any differences between the variables.

The small autosomal data was split into two models (elimination and non-zero) as described in 3.8 Data Analysis. For the elimination model, there were no statistically significant associations with detergent group, method, or soak condition (Table 4). The confidence intervals were also wide, indicating low precision. When the residual concentrations were evaluated, there were also no statistically significant associations with detergent group, method, or soak condition (Table 4). This can also be seen in Figure 7, where none of the comparisons varied much from the baseline at 1.0.

Table 4. Hurdle model with two-part regression analysis of small autosomal DNA elimination and residual concentration for Specific Aim 1.

Comparison	Elimination† OR (95% CI)	*p*-value	Residual Concentration‡ GMR (95% CI)	*p*-value
Detergent Group				
dry cleaning vs. low control	0.25 (0.02-2.32)	0.218	1.97 (0.29-13.5)	0.477
QAC vs. low control	0.44 (0.05-3.64)	0.437	2.04 (0.29-14.5)	0.465
miscellaneous vs. low control	0.44 (0.05-3.64)	0.437	3.17 (0.46-22.0)	0.234
high control vs. low control	0.14 (0.001-2.29)	0.182	2.26 (0.27-18.6)	0.438
Method				
swab vs. control	2.56 (0.38-27.9)	0.338	0.73 (0.21-2.51)	0.611
M-vac vs. control	2.56 (0.38-27.9)	0.338	0.39 (0.11-1.35)	0.131
Soak				
soak vs. no soak	1.33 (0.30-6.39)	0.707	0.52 (0.19-1.45)	0.204

† Elimination Model: Odds ratios (OR) derived from a penalized (Firth) logistic regression, modeling the probability of complete small DNA elimination. (penalized likelihood ratio test: $p = 0.832$)

‡ Residual Concentration Model: Geometric mean ratios (GMR) obtained by exponentiating coefficients from a linear regression model, fit to log-transformed positive small DNA measurements. (global F-test: $F(7, 33) = 0.77$, $p = 0.615$)

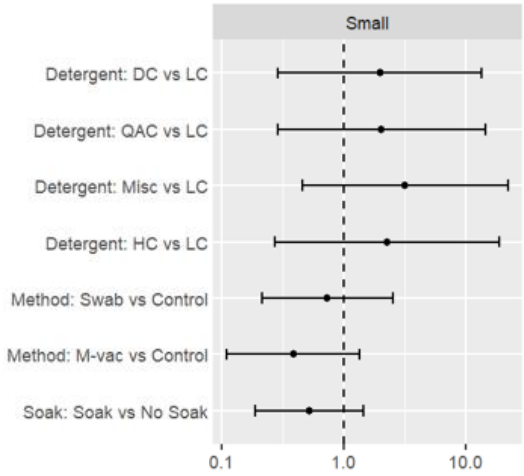

Figure 7. Adjusted geometric mean ratios (log scale) for small autosomal residual DNA concentration for Specific Aim 1.

4.1.3 Male Specific DNA

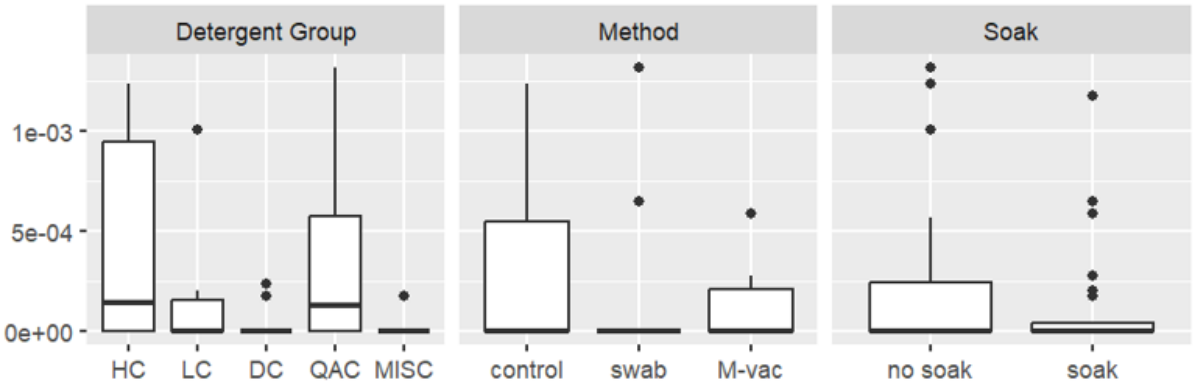

Figure 8. Boxplot of Y DNA concentrations for Specific Aim 1.

Although there were a large number of zeros for the Y DNA concentrations of Specific Aim 1, there were some visual differences in the boxplots of the Y DNA concentrations (Figure 8). The concentrations were still incredibly low despite the visual differences as shown in Table 2.

The Y data was split into two models (elimination and non-zero) as described in 3.8 Data Analysis. In the elimination model, none of the variables (detergent group, method, soak condition) had any significant p-values associated with them (Table 5). For the non-zero model, there were no predictors that actually achieved significance (Table 5). However, there was one predictor that approached statistical significance with a p-value of 0.056. This was the geometric mean ratio for the comparison of M-Vac™ to the control swab of the washing machine drum. The M-Vac™ was associated with lower Y DNA concentrations (Figure 9).

It should be noted that in the non-zero model, there was very little data that was a non-zero value. Only fourteen values were non-zero values, and this limited sample size limited the precision of the model. However, there are some interesting trends to be seen in where the non-zero concentrations came from. Out of the fourteen non-zero variables, six of the values came

from the QAC Group. Only two values came from the Dry Cleaning Group, and one value came from the Miscellaneous Group of detergents.

Table 5. Hurdle model with two-part regression analysis of Y DNA elimination and residual concentration for Specific Aim 1.

	Elimination†		Residual Concentration‡	
Comparison	OR (95% CI)	*p*-value	GMR (95% CI)	*p*-value
Detergent Group				
dry cleaning vs. low control	2.38 (0.27-22.2)	0.424	0.70 (0.20-2.44)	0.513
QAC vs. low control	0.53 (0.06-3.64)	0.520	1.00 (0.36-2.77)	0.992
miscellaneous vs. low control	4.40 (0.44-62.1)	0.206	0.56 (0.11-2.79)	0.411
high control vs. low control	0.53 (0.05-4.87)	0.574	1.44 (0.49-4.26)	0.445
Method				
swab vs. control	2.92 (0.56-19.7)	0.210	1.31 (0.41-4.21)	0.589
M-vac vs. control	0.58 (0.12-2.47)	0.459	0.41 (0.16-1.03)	0.056
Soak				
soak vs. no soak	1.54 (0.42-5.99)	0.511	0.87 (0.39-1.94)	0.690

† Elimination Model: Odds ratios (OR) derived from a penalized (Firth) logistic regression, modeling the probability of complete Y DNA elimination. (penalized likelihood ratio test: $p = 0.148$)

‡ Residual Concentration Model: Geometric mean ratios (GMR) obtained by exponentiating coefficients from a linear regression model, fit to log-transformed positive Y DNA measurements. (global F-test: $F(7, 6) = 3.87$, $p = 0.060$)

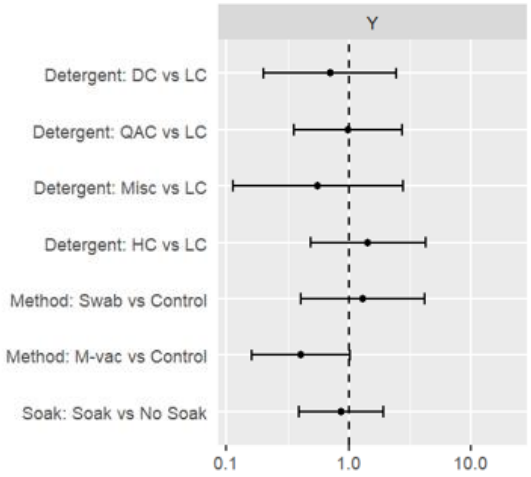

Figure 9. Adjusted geometric mean ratios (log scale) for Y residual DNA concentration for Specific Aim 1.

4.1.4 Bacterial Colony Counts

Swabs were taken from the collars of the laundered cotton shirts in order to determine the bacteria left on the shirts after the laundry products were used. These findings were compared

with a worn shirt that had not been washed. The average colony count of the replicates was calculated, and that data can be found in Table 6 below. When the results were compared, it was seen that several of the agar plates didn't have a single colony grown. These instances were primarily seen with shirts that were not pre-soaked and followed the directions of adding the product to the rinse cycle of the washing machine. There was only one non-soak shirt that had bacteria grown on the agar plate, and that was the Tide™ Sport shirt. This was then found to be statistically significant (p-value = 0.037), and the soak method was shown to have lower odds of elimination than the no soak method (Table 7). The products where nothing ever grew were OxiClean™, Lysol™, Silver Satin 2+. No statistical significance could be associated with any laundry product group (Table 7). However, every laundry product was effective in removing bacteria, as they had less bacteria grow than the unwashed shirt. The product with the highest average bacteria count was Clorox™.

There was one outlier in the data, which was the shirt soaked in the Tide™ Sport product. It had an average bacteria colony count of 157 bacteria, which is much higher than every other result. It is hypothesized that during the packaging for transportation to the lab, not enough air was allowed to circulate with the shirt, leading to more bacteria growing. It is for this reason that this outlier point was removed during analysis but is shown for completeness.

Table 6. Results from agar plating of bacterial swabs taken from the collars of the laundered cotton shirts.

Product	Condition	Average Colony Count
None	Control	1.33
Bleach	No Soak	0
Bleach	Soak	0.33
Clorox™	No Soak	0
Clorox™	Soak	0.67
OxiClean™	No Soak	0
OxiClean™	Soak	0
Tide™ 10X	No Soak	0
Tide™ 10X	Soak	0.33
Faultless Star Power	No Soak	0
Faultless Star Power	Soak	0.33
Silver Satin 2+	No Soak	0
Silver Satin 2+	Soak	0
Lysol™	No Soak	0
Lysol™	Soak	0
Tide™ Sport	No Soak	0.33
Tide™ Sport	Soak	157

Table 7. Elimination logistic model for bacterial colonies grown from shirt samples.

	Elimination†	
Comparison	OR (95% CI)	*p*-value
Laundry product Group		
DC vs. low control	3.33 (0.08-626)	0.522
QAC vs. low control	3.33 (0.08-626)	0.522
miscellaneous vs. low control	3.33 (0.08-626)	0.522
Soak		
soak vs. no soak	0.09 (0.001-0.87)	0.037

† Elimination Model: Odds ratios (OR) derived from a penalized (Firth) logistic regression, modeling the probability of complete bacterial colony elimination. (penalized likelihood ratio test: $p = 0.075$)

4.2 Specific Aim 2: Evaluation of DNA Transfer in a Washing Machine

The results of the overall DNA concentrations for Specific Aim 2 are shown in Table 8 below. There is average DNA concentration for large, small, and Y DNA along with standard deviation and the number of samples that had no detectable DNA. In the large autosomal DNA

and small autosomal DNA, there were no samples with no detectable DNA. In the Y DNA, there were some samples with no detectable DNA, approximately 58%. It was seen that even when there was a non-zero value, the DNA concentrations were very low (Table 8).

Table 8. Descriptive statistics for raw DNA concentrations for Specific Aim 2.

		Large DNA			Small DNA			Y DNA		
	n	mean	s.d.	zeros†	mean	s.d.	zeros†	mean	s.d.	zeros†
Detergent Group										
low control	6	0.00065	0.00041	0	0.00143	0.00061	0	0.00023	0.00037	3
QAC	12	0.00440	0.00380	0	0.00508	0.00553	0	0.00058	0.00104	5
miscellaneous	12	0.00822	0.01145	0	0.00612	0.00829	0	0.00005	0.00018	11
high control	6	0.00865	0.00529	0	0.00896	0.00483	0	0.00055	0.00080	2
Method										
control	12	0.00301	0.00457	0	0.00436	0.00595	0	0.00020	0.00036	9
swab	12	0.00466	0.00353	0	0.00403	0.00342	0	0.00016	0.00020	6
M-vac	12	0.00960	0.01125	0	0.00800	0.00828	0	0.00067	0.00114	6
Drops										
1 drop	18	0.00526	0.00873	0	0.00583	0.00777	0	0.00012	0.00024	12
3 drops	18	0.00625	0.00659	0	0.00560	0.00461	0	0.00057	0.00095	9

† Number of samples with no detectable DNA

4.2.1 Large Autosomal Data

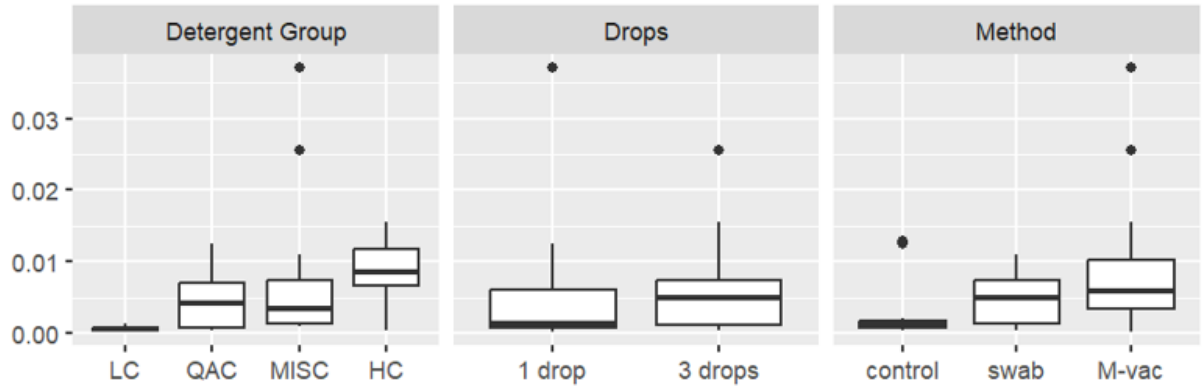

Figure 10. Boxplot for large autosomal DNA for Specific Aim 2.

The large autosomal DNA concentrations were fairly low but still showed some visual differences when the boxplots for each variable were evaluated (Figure 10). The large autosomal data was not split into two models as described in Section 3.8 Data Analysis because there were no zero values in this data set. Only the linear regression of the model using the log-transformed data was utilized, and this model was seen to be statistically significant (p-value = 0.005). In this

model, there were differences in all of the detergent groups compared to the low control of bleach (Table 9). There were significantly higher DNA concentrations in the QAC Group (p-value = 0.010), miscellaneous group (p-value = 0.001), and high control detergent of Tide™ Sport (p-value = 0.001). This can also be seen in the geometric mean ratios seen in Figure 11. Furthermore, the comparison between the M-Vac™ and the control swabs that were taken of the drum had a statistically significant association with a p-value of 0.025 (Table 9). The M-Vac™ was associated with having higher large autosomal DNA concentrations than control swabs of the washing machine drum (Figure 11). The amount of blood input (Three drops versus 1 drop) was not seen to have a statistically significant difference (p-value = 0.206).

Table 9. Linear regression analysis of log-transformed large autosomal DNA concentration for Specific Aim 2.

	Residual Concentration‡	
Comparison	GMR (95% CI)	*p*-value
Detergent Group		
QAC vs. low control	4.67 (1.49-14.7)	0.010
miscellaneous vs. low control	7.23 (2.30-22.7)	0.001
high control vs. low control	10.6 (2.83-39.7)	0.001
Method		
swab vs. control	2.03 (0.80-5.18)	0.131
M-vac vs. control	2.93 (1.15-7.47)	0.025
Drops		
3 drops vs. 1 drop	1.62 (0.76-3.47)	0.206

‡ Residual Concentration Model: Geometric mean ratios (GMR) obtained by exponentiating coefficients from a linear regression model, fit to log-transformed positive large DNA measurements. (global F-test: $F(6, 29) = 3.98$, $p = 0.005$)

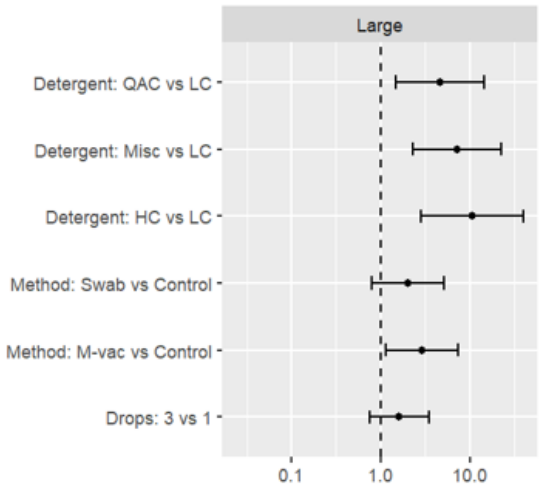

Figure 11. Adjusted geometric means ratios (log-scale) for large autosomal residual DNA concentration for Specific Aim 2.

4.2.2 Small Autosomal Data

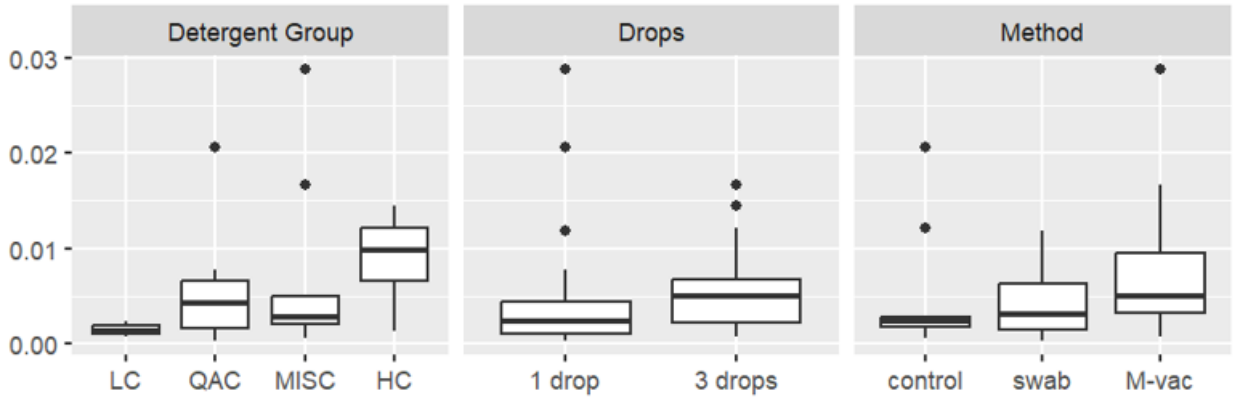

Figure 12. Boxplot of small autosomal DNA for Specific Aim 2.

Although there were no zeros for the small autosomal DNA concentrations, the DNA concentrations were still quite low. However, the boxplots of the small autosomal DNA concentrations show some visual differences between the variables (Figure 12).

The small autosomal data was not split into two models as described in Section 3.8 Data Analysis because there were no zero values in this data set. Only the linear regression model with the log-transformed data was considered. In the non-zero model, the only statistically significant comparison was with the detergent groups (Table 10). The high control of Tide™

Sport was found to be significantly higher than the low control of bleach (Figure 13). Another comparison of the low control of bleach approached statistical significance with a p-value of 0.064 (Table 10). It was seen that the Miscellaneous detergent group was almost found to be significantly greater than the low control of bleach (Figure 13). Neither the drops condition nor the collection method yielded any significant results (Table 10).

Table 10. Linear regression analysis of log-transformed small autosomal DNA concentration in Specific Aim 2.

	Residual Concentration‡	
Comparison	GMR (95% CI)	*p*-value
Detergent Group		
QAC vs. low control	2.17 (0.78-6.06)	0.133
miscellaneous vs. low control	2.63 (0.94-7.34)	0.064
high control vs. low control	5.38 (1.64-17.6)	0.007
Method		
swab vs. control	1.01 (0.44-2.34)	0.976
M-vac vs. control	1.78 (0.77-4.12)	0.169
Drops		
3 drops vs. 1 drop	1.68 (0.85-3.33)	0.132

‡ Residual Concentration Model: Geometric mean ratios (GMR) obtained by exponentiating coefficients from a linear regression model, fit to log-transformed positive large DNA measurements. (global F-test: $F(6, 29) = 2.28$, $p = 0.064$)

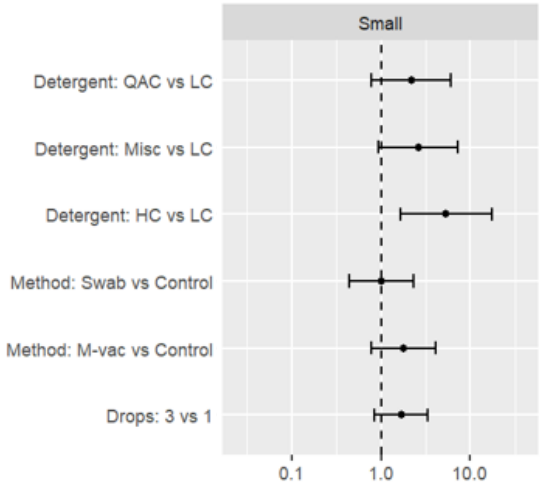

Figure 13. Adjusted geometric mean ratios (log-scale) for small autosomal residual DNA concentration for Specific Aim 2.

4.2.3 Male Specific DNA

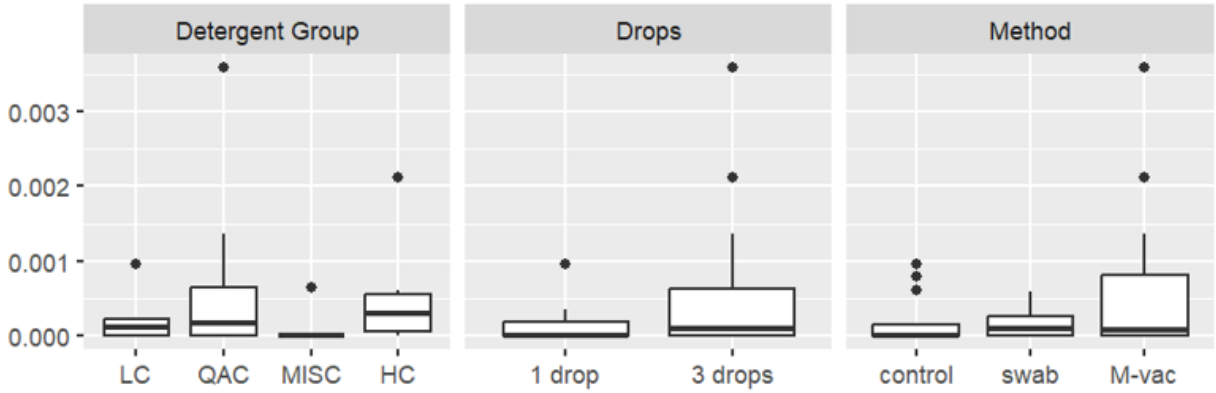

Figure 14. Boxplot for Y DNA for Specific Aim 2.

Although there were several zeros present in the Y DNA concentration data, there were still samples with non-zero values. When these concentrations are considered, there are some minute visual differences in the boxplots of the concentration data for the variables studied (Figure 14).

The Y DNA concentration data was split into two models (elimination and non-zero) as described in Section 3.8 Data Analysis. In the elimination model, it was seen that there were no statistically significant associations between any of the variables, detergent group, collection method, or drops condition (Table 11). There was only one comparison that approached significance with a p-value of 0.062. It was seen through the geometric mean ratio that the Miscellaneous detergent group was almost associated with higher odds of elimination than the low control of bleach (Table 11).

In the non-zero model, there were no statistically significant variables (Table 11 and Figure 15). However, something interesting was revealed when looking at where the non-zero values originated from. There were only fifteen non-zero values for the Y DNA data for Specific Aim 2. Of those fifteen values, seven of them came from the QAC Group and one of them came

from the Miscellaneous Group. It is interesting to see such an imbalance in the occurrences of the non-zero values.

Table 11. Hurdle model with two-part regression analysis of Y DNA elimination and residual concentration for Specific Aim 2.

Comparison	Elimination† OR (95% CI)	*p*-value	Residual Concentration‡ GMR (95% CI)	*p*-value
Detergent Group				
QAC vs. low control	0.72 (0.10-4.97)	0.738	0.77 (0.13-4.51)	0.738
miscellaneous vs. low control	7.88 (0.91-111)	0.062	0.42 (0.02-7.12)	0.497
high control vs. low control	0.54 (0.05-4.83)	0.578	0.79 (0.12-5.31)	0.782
Method				
swab vs. control	0.31 (0.04-1.76)	0.188	0.48 (0.11-2.15)	0.290
M-vac vs. control	0.31 (0.04-1.76)	0.188	1.17 (0.26-5.34)	0.815
Drops				
3 drops vs. 1 drop	0.45 (0.09-1.88)	0.277	2.96 (0.72-12.2)	0.116

† Elimination Model: Odds ratios (OR) derived from a penalized (Firth) logistic regression, modeling the probability of complete Y DNA elimination. (penalized likelihood ratio test: $p = 0.075$)

‡ Residual Concentration Model: Geometric mean ratios (GMR) obtained by exponentiating coefficients from a linear regression model, fit to log-transformed positive Y DNA measurements. (global F-test: $F(6, 8) = 1.44$, $p = 0.310$)

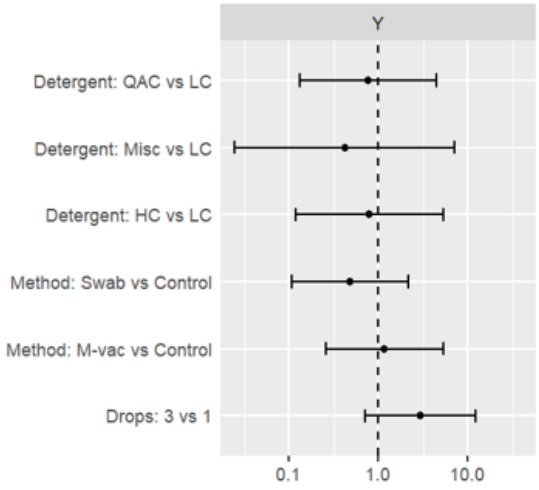

Figure 15. Adjusted geometric mean ratios (log-scale) for Y residual DNA concentration for Specific Aim 2.

4.3 DNA Profile Quality

After quantitation, eleven high concentration samples and two low concentration samples were chosen for amplification and capillary electrophoresis in order to obtain DNA profiles. Of

those, eleven electropherograms were successfully able to be analyzed. Both of the low concentration samples and nine of the high concentration samples had electropherograms that were able to be analyzed. Seven of the electropherograms came from samples collected with the M-Vac™, and the other four were collected with swabs. The high concentration samples were washed with laundry products OxiClean™, Tide™ Sport, and Tide™ 10X (Table 12).

Table 12. List of samples that produced electropherograms through capillary electrophoresis with attributes of sample.

Type of Concentration	Sample	Laundry Product	Collection Method	3 Drops vs 1 Drop	Soak vs No Soak
Low	LSS3	Lysol™	Swab	NA	Soak
High	O3V2	OxiClean™	M-Vac™	3 Drops	NA
High	O3V3	OxiClean™	M-Vac™	3 Drops	NA
High	OSC	OxiClean™	Swab	NA	Soak
Low	OSV1	OxiClean™	M-Vac™	NA	Soak
High	R1V1	Tide™ Sport	M-Vac™	1 Drop	NA
High	R3S2	Tide™ Sport	Swab	3 Drops	NA
High	R3V1	Tide™ Sport	M-Vac™	3 Drops	NA
High	T1V2	Tide™ 10X	M-Vac™	1 Drop	NA
High	T3S2	Tide™ 10X	Swab	3 Drops	NA
High	T3V1	Tide™ 10X	M-Vac™	3 Drops	NA

In terms of quality, the electropherograms were good quality with little artifacts present (Figure 16). There was occasionally pull up from other dye channels or stutter peaks present, but

they were easily distinguishable from the actual called alleles in the electropherograms (Figure 17). The one caveat is that one of the capillaries on the genetic analyzer was having issues after much troubleshooting, which often caused issues in the red dye channel, but this was an issue that persisted across almost all of the samples due to it being the fault of the genetic analyzer itself (Figure 18). Each electropherogram was determined to be a single source profile and was the same profile in each sample. Each profile was almost a full profile apart from T3V1, which had several instances of allelic dropout, where no information was available at a locus (Figure 19). When it came to comparing the electropherograms to the known DNA profiles of the participants, it was found that each participant could be excluded from the electropherograms of the samples. Next, household members of where the washing machine was kept were compared to the electropherograms of the samples. It was found that the DNA profile of a household member was consistent with the DNA profile found in the samples.

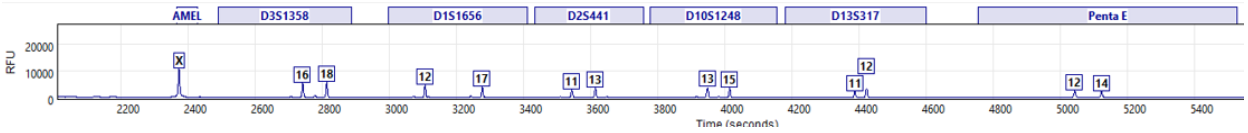

Figure 16. Example of clear electropherogram from sample O3V2.

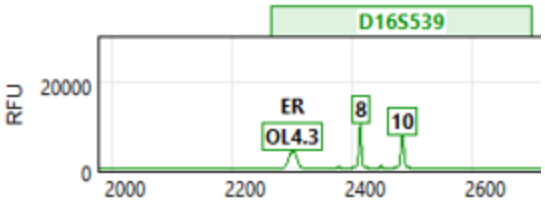

Figure 17. Example electropherogram loci from sample O3V3 where there was an artifact present that appeared as an off-ladder allele and was consistent with a dye blob.

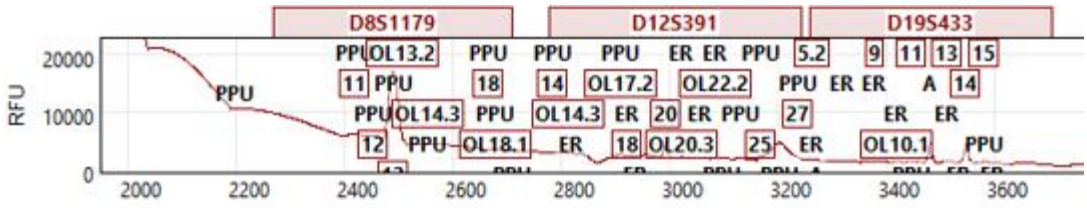

Figure 18. Example electropherogram from sample T3S2 where the capillary was malfunctioning and causing lots of noise on the electropherogram.

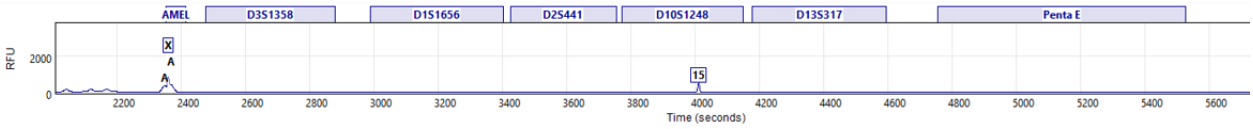

Figure 19. Example electropherogram from T3V1 where significant allelic drop-out occurred and limited information was available.

4.4 Control Swabs

As mentioned earlier, control swabs were taken of the drum of the washing machine after a cleaning regimen was completed in between the washing of each sample. It was found that there was actually DNA that was collected by these control swabs (Table 13). The DNA concentrations found were mostly in the large and small autosomal regions. The Y concentrations were often zeros (Table 13). The concentrations that were non-zero were often low in their concentrations, but some samples were higher than the others. Sample OSC (OxiClean™ Soak Control) had a high enough concentration that it was one of the samples chosen for genetic profiling (Table 12). The sample had sufficient enough DNA to be able to produce an electropherogram. This once again shows that the cleaning regimen chosen for this thesis project was not sufficient to prevent leftover DNA from being present.

Table 13. DNA concentrations for the control swabs that were taken from the drum of the washing machine.

Sample Name	Large Concentration (ng/mL)	Small Concentration (ng/mL)	Y Concentration (ng/mL)
B$C	0.001577	0.001733	0.001007
B1C	0.001285	0.00131	0.000962
B3C	0.00839	0.001988	0
BSC	0.000204	0.000333	0
F$C	0.000119	0	0
FSC	0	0.000411	0
L$C	0.00151	0.0022	0.000568
L1C	0.000635	0.00054	0
L3C	0.000337	0.001803	0
LSC	0	0.000366	0
O$C	0.03003	0.036827	0
O1C	0.001174	0.002788	0
O3C	0.000987	0.002118	0
OSC	0.010667	0.010517	0
R$C	0.009627	0.017501	0.001235
R1C	0.000412	0.001269	0
R3C	0.012909	0.012164	0.000599
RSC	0.004868	0.007081	0.00117
S$C	0	0.001597	0
SSC	0.000342	0.000949	0
T$C	0.000512	0.001598	0
T1C	0.001279	0.002696	0
T3C	0.001674	0.002541	0
TSC	0.000207	0.000422	0
X$C	0.000754	0.001555	0.00054
X1C	0.012579	0.020656	0
X3C	0.002003	0.002492	0.000785
XSC	0.000851	0.001607	0

4.5 Summary of Laundry Products

Figure 20 below shows the average DNA quantitations for all three DNA targets across both specific aims. There are some interesting trends to be seen when it comes to assessing the laundry products tested in this study. Firstly, it was seen that the Y DNA concentrations were considerably lower than the small and large autosomal target concentrations. There were several products that had higher DNA quantitations, such as the Tide™ 10X and the OxiClean™, which

were comparable to the high control of the Tide™ Sport product. Then there are several products that had lower DNA quantitations, such as Faultless Star Power, Silver Satin 2+, and Lysol™ which were more comparable to the low control of bleach. Faultless Star Power seems to have very high DNA quantitations in the small autosomal target, but this is caused by an extreme outlier in one replication. It should be evaluated through the other targets (large autosomal and Y) that show more consistent low concentrations of DNA.

However, it should be noted that this experimental design produced low DNA concentrations as seen in the Y-axis (Figure 20) and with the statistical analysis done in Sections 4.1 and 4.2. With this low-level DNA came variability that resulted in large standard deviations. It was for this reason that standard deviation error bars were not included in Figure 20 to preserve comprehension of the figure and its trends. Caution should be used when assessing these results, and more replications should be done in future studies to confirm the trends observed in this study.

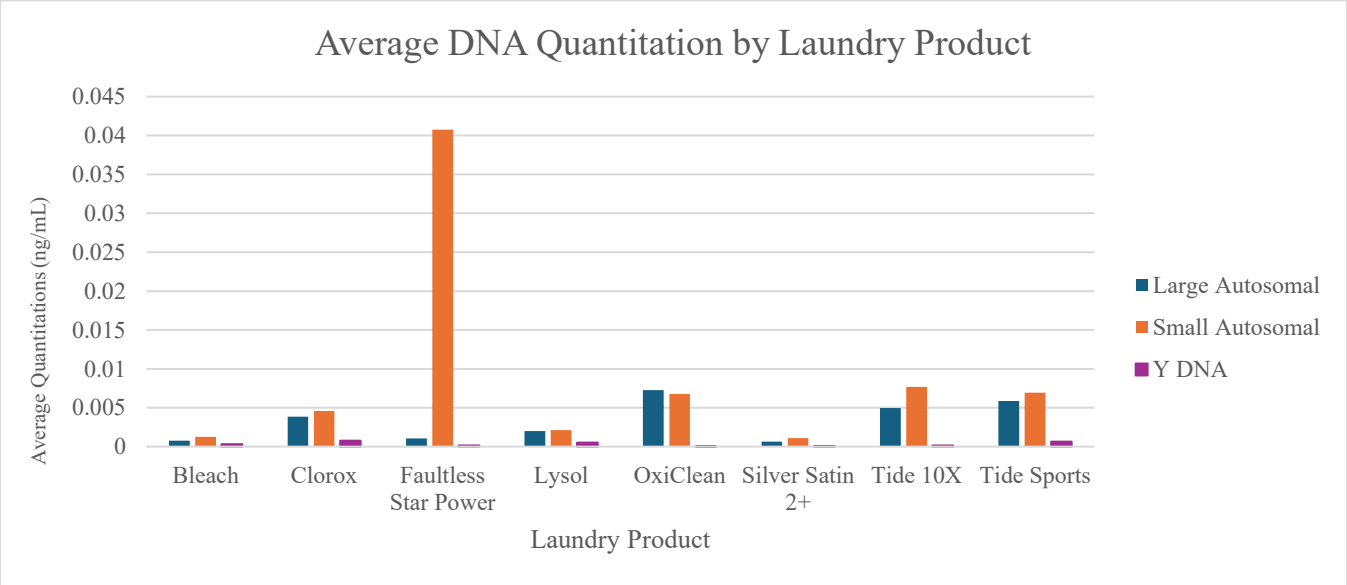

Figure 20. Summary of DNA quantitations (Large autosomal, small autosomal, and Y) split by laundry product across both specific aims.

5. Discussion

5.1 Specific Aim 1

The detergent group variable was seen to not have any statistical analysis when it came to DNA elimination or DNA concentration in Specific Aim 1. All of the models for large autosomal, small autosomal, and Y DNA found that there were no statistically significant differences between the Dry Cleaning Group, QAC Group, Miscellaneous Group, or the High Control when compared to the Low Control of bleach. This is quite an unusual finding, as most other studies in the literature have found a laundry additive to either be better or worse at removing DNA from clothing (Edler et al., 2017; Thabet et al., 2018). However, it can be seen from the data that the confidence intervals calculated are quite wide and imprecise (Tables 3, 4, & 5). The DNA concentrations are very low and often overlap with one another as shown by the boxplots in Figure 4, 6, and 8. There were several instances of zeros in the data set for Specific Aim 1 (Table 2), which could contribute to the overlap and lack of significant associations.

The collection method of M-Vac™ and conventional swab had slightly more significant conclusions drawn from it. In the large DNA concentration dataset, the M-Vac™ was seen to have statistically significant lower levels of DNA when compared to the control swabs taken of the drum of the washing machine (Table 3). This same trend approached statistical significance in the Y DNA concentration dataset (Table 5) but was not seen in the small autosomal DNA dataset (Table 4). This is contrary to most of the literature about the M-Vac™. In most of the literature, the M-Vac™ does a very good job at collecting DNA from clothing even after other methods have been utilized (Blackmore et al., 2024; Vickar et al., 2018). However, it was seen in this study that there were some issues with collecting DNA from the T-shirts using the M-Vac™. When the sterile buffer was sprayed, it completely saturated the thin shirt almost immediately. Then, excess buffer was left around the shirt. A nonporous surface was placed underneath the

shirt to attempt to catch the excess buffer and collect this excess buffer in addition to what was in the T-shirt. However, it was quite difficult to collect the excess buffer, and the nonporous surface was often seen to be damp after the M-Vac™ was used. It could be that this loss in combination with the low levels of DNA present after washing led to lower levels of DNA being collected with the M-Vac™.

The soak condition variable was never found to be statistically significant or even approaching significant in any of the models evaluating large autosomal, small autosomal, or Y DNA concentrations. From these results, it can be seen that it does not matter when the laundry additive is added to the shirt when it comes to DNA persistence. Most studies involving laundry additives don't focus on how the laundry additives are applied to the evidence, just the presence or absence of a type of laundry additive. Therefore, it is quite interesting to find that application doesn't necessarily matter as much as the simple use of a laundry additive.

When bacterial counts were assessed, it was once again found that the laundry additives were efficient at removing the bacteria from the shirts compared to the unwashed shirt control (Table 6). This is in agreement with the studies of laundry sanitizers affecting bacterial growth, which show that laundry sanitizers are generally efficient at their purpose of removing bacteria from clothing (Farcas et al., 2019; Khalid Ijaz et al., 2022). Several interesting trends could be identified by simply looking at the average bacteria counts for each shirt. There were several additives in which bacteria never grew at all, including Lysol™, OxiClean™, and Silver Satin 2+. It was seen that the no soak shirts often didn't have any bacteria grow at all, with the exception of the No Soak Tide™ Sport shirt. It was seen that the shirts that were soaked had a significantly lower chance of elimination than the shirts where the laundry sanitizer was added into the rinse cycle (Table 7). This was unexpected, as it was thought that the shirts that were

soaked would have less bacterial growth than the shirts that were treated via the rinse cycle. There was one outlier in the data (Soak Tide™ Sport) which had an incredibly high bacterial count, potentially due to improper packaging, which led to this point being removed from analysis.

All in all, it was found that the laundry additives generally did a good job at removing DNA from clothing, as evidenced by the low average DNA concentration for all variable combinations (Table 2). There was no evidence that laundry detergent group or soak versus no soak showed any statistically significant differences in this specific aim. There was some evidence that the M-Vac™ was associated with lower levels of DNA collected from clothing, potentially due to the issues that occurred with the thin nature of the shirt. In terms of bacterial count on the clothing, the shirts treated with the laundry additive had a lower bacterial count than the unwashed shirt. The no soak condition was seen to have significantly higher chances of eliminating bacterial growth than the soak condition. However, there were several zeros in the dataset that make statistical analysis difficult.

5.2 Specific Aim 2

The detergent group variable was seen to have the most statistically significant associations with DNA concentration after laundering. The QAC Group, Miscellaneous Group, and High Control were all seen to be significantly higher than the Low Control when the large autosomal DNA was evaluated. This significance was somewhat lost in the small autosomal DNA concentration models, where only High Control and Low Control were significantly different from one another. However, the Miscellaneous Group approached being significantly different from the Low Control. When the Y DNA was evaluated, the Miscellaneous Group approached being significantly more likely to cause elimination compared to the Low Control.

This means that although the tested laundry products were somewhat efficient at preventing DNA transfer during the washing cycle, as evidenced by the extremely low average DNA concentration for each interaction (Table 8), it was still inferior to using the laboratory standard of bleach. However, it was seen that these laundry additives often had lower DNA concentrations than the High Control of a regular laundry detergent Tide™ Sport (Figures 10 and 12).

Other studies have found conflicting evidence about the effectiveness of laundry additives versus conventional laundry detergents, with one study finding that stain remover did better than regular detergent (Thabet et al., 2018) and another study finding that stain remover did not do well at removing bloodstains from clothing (Edler et al., 2017). Limited studies have been conducted on laundry additives with QACs, which is the active ingredient in many laundry sanitizers. One study utilized a hygiene rinse, which also utilizes QACs. This study only looked at the removal of bloodstains from clothing but found that the hygiene rinse did not do well at removing bloodstains from clothing (Edler et al., 2017). This is in accordance with what this project found where the QAC containing detergents were found to be significantly higher than the bleach low control (Table 9).

The collection method variable (M-Vac™ and conventional swab) was not often seen to be statistically significant. The M-Vac™ was only seen to have significantly higher DNA concentrations than the control swabs taken of the drum of the washing machine when the large autosomal DNA was evaluated (Table 9). This shows that the M-Vac™ collects a significantly larger amount of DNA transferred during the washing process. It is interesting that the M-Vac™ is found to have significantly higher DNA in this specific aim when the M-Vac™ was found to collect significantly lower DNA concentrations than the control swabs in Specific Aim 1. It has been shown from the research done on the M-Vac™ that the attributes of the evidence being

sampled significantly affect the DNA concentration that is collected (Blackmore et al., 2024; Garrett et al., 2014; McLamb et al., 2020; Vickar et al., 2018). In Specific Aim 2, the fabric that was being sampled was a washcloth whereas the fabric being sampled in Specific Aim 1 was a T-shirt. While both fabrics were 100% cotton, the thickness of the two fabrics were quite different. The cotton shirt was thin compared to the washcloth. During the collection process using the M-Vac™, the buffer soaked into the thin shirt almost immediately, especially when compared to the washcloth, which caused some issues with collection. It could be that the thickness of the weave is to account for the different trends seen across the Specific Aims when it comes to the effectiveness of the M-Vac™.

When the quantity of blood was considered, it was never seen to have a significant association with DNA elimination or DNA concentration (Tables 9, 10, & 11). Original washcloths were either spotted with three drops of blood or one drop of blood and washed. None of the models ever found a statistical difference between the two quantities when large, small, or Y DNA concentrations were evaluated. It would make logical sense that if there was more biological material inputted onto a piece of evidence, then more biological material would be available for transfer. However, a potential explanation for this could be that simply both quantities were too small or too similar to one another. Each “drop” was chosen to be 25 µL, which means that the real difference between the two quantities was 50 µL. It could potentially be that this simply isn’t enough biological material to make a real difference in DNA transfer during a washing cycle. Another possible explanation is that since the washing machine only had the two washcloths present during one washing cycle, that the blood got diluted and erased any differences between the two quantities. One study looking at bloodstain transfer in a washing

machine found that when the washing machine was less filled, the original stain washed better but any transfer to other pieces of fabric were diluted (Hofmann et al., 2019).

All in all, there were more associations with DNA concentration and variables studied in Specific Aim 2. Several more statistically significant associations between detergent group and DNA concentration were found in Specific Aim 2 compared to Specific Aim 1 and one statistically significant association was found in the collection method variable. This increase in statistical significance is most likely due to the fact that were fewer zero values in the data set of Specific Aim 2. In fact, the small and large autosomal sets had no zeros at all and were able to only use the linear regression model of the log-transformed data instead of needing the elimination model in addition to the linear regression model. It is potentially true that there are less zeros in Specific Aim 2 because Specific Aim utilizes blood versus Specific Aim 1 which utilizes DNA transferred to a shirt while exercising. This is most likely going to be the form of epithelial cells. The number of epithelial cells that transfer to clothing can be variable when compared to a set volume of blood spotted on fabric.

5.3 DNA Profile Quality

A subset of the samples was chosen for genetic analysis through electrophoresis from both of the specific aims. Three samples were shirts from Specific Aim 1 and eight samples were washcloths from Specific Aim 2. Out of the eleven electropherograms that were able to be produced through capillary electrophoresis, it was seen that the majority of profiles were full, single source profiles. The one exception was T3V1, which was a partial profile due to numerous instances of allelic dropout (Figure 19). There were occasional instances of artifacts, such as pull-up and dye blobs, in the electropherograms (Figure 17). It should be noted that they were easy to distinguish as artifacts and were easily identified and notated as artifacts.

However, the biggest issue with the DNA profiles was the source of the DNA profiles. When the electropherograms were compared with known electropherograms from the participants in the thesis project. It became apparent that the participants were able to be excluded from the sample electropherograms. Therefore, the known DNA profiles of the household members of the washing machine were obtained and analyzed. From there, one of the household members was seen to be consistent with the DNA profiles obtained from the samples. This is quite surprising since studies looking at background DNA on washing machine drums often find little to no DNA present on uncleaned washing machines, both public (Voskoboinik et al., 2018) and private (van den Berge et al., 2016).

The finding of household member DNA in the samples is despite the extensive cleaning methods that were utilized on the washing machine to prevent carryover DNA from the household and between the samples. Therefore, the findings of this thesis project need to be evaluated with caution due to the presence of the household member DNA in the samples. The household member was found to have XX chromosomes at Amelogenin and both the participants had XY chromosomes at Amelogenin. It is important to note that the household member found in the subset of samples tested was the individual conducting the washing machine research in this study. The household member was also related to the participant from Specific Aim 1 and would therefore share half of their DNA with the participant. Consequently, it would be more beneficial to evaluate the findings of the Y data, since it would be more likely that it came from the actual participants. A further study would need to be done to find a cleaning methodology that would reduce DNA carryover. However, these findings do bring up another interesting avenue of thought where the washing machine surfaces themselves are a source of potential DNA transfer, not just the washing cycle.

This need for further cleaning is also reiterated by the control swabs that were found to have DNA concentrations on them (Table 13). While several of them were fairly low DNA concentrations, there were still several DNA concentrations that were high. One sample had a high enough concentration to be chosen for genetic analysis through capillary electrophoresis. This sample did indeed produce an electropherogram, and it was shown to be the household member of the participant.

5.4 Limitations

5.4.1 Private Washing Machine

The main limitation of this thesis project is the location of the washing machine. The washing machine was located in a private home that was shared by the people that lived there. This choice was made intentionally to avoid the possible outside factors that would come from using a public washing machine that was available to anyone. Because of this, the household members had access to the room with the washing machine and had been there in the past. Therefore, DNA that was not the intended participants was found on the T-shirts and washcloths in the thesis project. This is despite the several cleaning steps that were taken to avoid this. The washing machine itself was cleaned with washing machine cleaner and a sanitizing cycle before the study started, in between each sample, and after the study concluded. After the sanitizing cycle, the drum of the washing machine was physically wiped out with a disinfecting wipe. When DNA profiles were evaluated, it was found that a household member, who was working on the project, was the DNA profile that was present on all of the samples analyzed.

5.4.2 Packaging of the First Shirt for Bacterial Assessment

One other limitation is a particular data point for the bacteria colony assessment (Section 4.1.4). One agar plate had particularly high bacteria counts compared to every other agar plate assessed. It is suspected that during the packaging of the T-shirt that was sampled, it was

packaged in plastic too tightly. This meant that the T-shirt was not able to have access to the air and perhaps had higher bacterial counts due to packaging rather than the laundry product that was being investigated. It is for that reason that analysis was done without that particular agar plate to see if trends were able to be identified without the outlier in the analysis.

5.4.3 Number of Replicates

For each combination of variables, there were only three analytical replicates done. Since this thesis project worked with laundered fabric, the DNA present was often in low quantities. This means that it can be hard to reach statistical significance. This can be seen by the fact that in several instances comparisons were close to significance, but few variables were of actual statistical significance. It would be beneficial to increase the number of replicates in order to see if any trends move towards statistical significance. This would also potentially lead to a smaller ratio of zeros to non-zero values, which would also help in the statistical analysis. More replicates would allow trends identified here to be confirmed or to identify new trends in further studies.

5.4.4 Issues with the Genetic Analyzer

There were several issues with the genetic analyzer, which lead to some troubles with the resulting electropherograms that established actual DNA profiles of the samples in both specific aims. Even after copious troubleshooting, it was only possible to get eleven profiles out of the thirteen samples put forward for genetic analysis. It cannot be ruled out that the failure of the two samples to produce an electropherogram is due to the genetic analyzer itself and not issues with the samples. Where the genetic analyzer was able to produce electropherograms, there were issues with a capillary that produced lots of noise and artifacts, primarily in the first few loci of the red dye channel. This means that almost every electropherogram was missing a couple of loci

due to the capillary malfunction. There were only two sample electropherograms that had genetic information in those specific locations.

5.5 Future Directions

5.5.1 Increase the Number of Replicates

The main takeaway from this thesis project is to increase the number of replicates per combination of variables. In this thesis project, there were three replicates per combination. In a future endeavor, it would be important to increase the replicates for each combination. During statistical analysis, there were several variables that were only close to significance. If more replicates were included, perhaps somewhere in the range of six to ten, it could push those specific variables to significance. From there, it would be possible to look at the pairwise comparisons and determine where the differences actually are and if there are some products or methods that are better than others.

5.5.2 Change Cleaning Regime

In this thesis project, it was seen that the cleaning regime of the washing machine was not sufficient to remove DNA on the control swabs taken of the drum of the washing machine. The cleaning regime for this thesis project was the washing machine's own sanitizing cycle with a washing machine tablet. Finally, the drum of the washing machine was physically wiped out with a disinfecting wipe before the control swab was taken. This regime was done at the beginning of the data collection and in between each sample wash.

There are several different ways that the cleaning regime could be troubleshooted. Firstly, a different washing machine cleaner could be utilized. There are several washing machine cleaners on the market, and the cleaner chosen for this regimen (Tide™) was based on

availability. Perhaps a different washing machine cleaner would work better at removing DNA from the drum of the washing machine, or bleach could even be used in substitution.

Another step of the cleaning process that should be further explored is the physical cleaning of the drum with a disinfecting wipe. Perhaps the mechanical motion of wiping the drum released DNA that was embedded in hard-to-reach crevices of the machine and then collected by the control swab. This is a step that would need to be investigated more for its usefulness in cleaning the drum of the washing machine.

5.5.3 Change Selection of Participants

For ease of analysis, one of the participants of this study was related to owner of the washing machine. For this reason, evaluating the DNA profiles from each sample was more challenging, as shared alleles had to be considered due to the presence of DNA from household members. In the future, it would potentially be beneficial to find participants unrelated to the owner of the washing machine if another private washing machine was utilized in a future study.

5.5.4 Explore Other Laundry Additives

In future studies, it would be beneficial to look at other laundry sanitizers or commercial laundry products. It was seen that the products tested in this thesis project did have some ability to remove DNA from fabrics, both DNA from wear and DNA from biological stains. The commercial dry-cleaning products were only used in Specific Aim 1 evaluating worn T-shirts, and not in Specific Aim 2 evaluating blood spotted on washcloths. More statistically significant detergent-based associations were found in Specific Aim 2 than in Specific Aim 1. Specifically, further testing of commercial laundry products should be utilized to see if statistically significant trends emerge. It would then be possible to see how those products fair in terms of comparing them to widely available brands such as Tide™, Clorox™, and Lysol™. Only two commercial

dry-cleaning products were investigated in this thesis project (Faultless Star Power and Silver Satin 2+), but there are many more that could be studied.

6. Conclusions

In this thesis project, DNA persistence and transfer in a washing machine were evaluated in the context of added laundry sanitizers. These sanitizers have become increasingly popular in use but remain underexplored in the scientific literature. Other research has shown that other laundry additives often have an effect on the DNA concentrations left on laundered fabric. This study looked at several variables: the laundry product (Specific Aims 1 & 2), the DNA collection method (Specific Aims 1 & 2), the amount of biological material initially left (Specific Aim 2), and whether the laundry product was applied as a pre-soak or as part of the rinse cycle of the washing process (Specific Aim 1). Specific Aim 1 also evaluated the bacterial colony presence on the fabric after washing with the laundry products.

It was found that detergent type was found to have a significant effect on the residual DNA concentration in Specific Aim 2. All of the detergent types studied had higher levels of DNA compared to the low control of bleach, which is commonly used in laboratories to remove DNA from clothing. There were some conflicting findings about the effectiveness of the M-Vac™ as a DNA collection tool. It was associated with lower DNA concentrations in Specific Aim 1 but was associated with higher DNA concentrations in Specific Aim 2. This could be due to the difference in thickness of the fabric studied in each specific aim, with a thin shirt being used in Specific Aim 1 and a washcloth being used in Specific Aim 2. The set of laundry sanitizer directions used and the initial input of blood were not seen to be statistically significant when it came to DNA concentrations. However, the set of laundry directions did seem to have an effect on bacterial presence. It was seen that the shirts that were treated via the rinse cycle were

significantly more likely to eliminate bacteria than shirts that were treated via a pre-soak before washing.

However, it should be noted that these findings should be evaluated with caution as there are limited replicates and precision associated with the statistical models. Additionally, DNA from a household member was found in several of the samples. The household member was determined to have XX chromosomes at Amelogenin, whereas both of the participants had XY chromosomes at Amelogenin. There was also DNA found on the control swabs that were taken from the drum of the washing machine. Therefore, it would be beneficial to pay more attention to the statistical associations from the Y DNA concentrations, since it would be more likely to be from the participants. Unfortunately, there are lots of zeros in the datasets for Y DNA concentrations which made statistical significance harder to achieve. However, some statistical trends were still seen in the Y DNA concentrations, such as M-Vac™ being associated with lower DNA concentrations in Specific Aim 1 and detergent type having a significant effect on potential elimination of DNA in Specific Aim 2.

There are a couple of limitations with this thesis project that should be addressed in future studies. Primarily, the finding of household DNA in the samples could be associated with the location of the washing machine or the cleaning methodology that was used to clean the washing machine itself. It would be important to either change the location of the washing machine to where only researchers had access or to change the cleaning methodology to account for a washing machine used by others in the past although the washing machine was not used by the household while the samples were being laundered. The participants could also be more carefully selected in the future to remove any association with the washing machine through access or relation to the owner of the washing machine. Additionally, the number of replicates

turned out to be a limitation. There were only three analytical replicates per combination of variables, which made statistical analysis difficult. It would be a good next step to increase replicate numbers in order to confirm statistical associations identified in this study, or to discover new significant trends not able to be identified here due to replicate size. Nonetheless, this thesis project adds to the literature about laundry sanitizers, a laundry product barely mentioned in the literature, and the M-Vac™, a collection method gaining more attention for its use on porous substrates.

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