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The Effects of Various Laundering Factors on the Recoverability of Dna

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THE EFFECTS OF VARIOUS LAUNDERING FACTORS ON THE RECOVERABILITY OF DNA

A thesis submitted in partial fulfillment of the
requirements for the degree of
Master of Science

By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Erin Lee (Berdanier) Houston ENTITLED The Effects of Various Laundering Factors On The Recoverability Of DNA BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master Of Science

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ABSTRACT

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The Effects Of Various Laundering Factors On The Recoverability Of DNA

Criminals have been documented to launder clothing in an attempt to hide evidence; however, there limited studies on this type of evidence manipulation. This study looked at: 1) the effects of eighteen laundry additives at diluted and undiluted strengths on human blood, 2) the effects of a delay between deposit and laundering, 3) the amount of recoverable DNA on laundered clothing with different deposited volumes of blood, and 4) the transfer of genetic material within a primary load and between primary/secondary and primary/tertiary loads. There was a reduction in volume of DNA for some laundry additives. Nevertheless, all genotyped samples were consistent with expected profiles. The results show that DNA can be transferred between a primary and a secondary load but not to a tertiary with the best locations for genetic transfer recovery being towels and socks. This study helps further the understanding and treatment of DNA on laundered clothing.

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Extra thanks go to the staff at the Ohio STEM Ability Alliance for all of their assistance both in and out of school. This research would not have been completed without their morale support and generous scholarship funding.

DEDICATION

I would like to dedicate this work to my husband, and my parents. Your daily encouragement kept me going to finish this program. Your love for me never ends, and I thank you for it.

CHAPTER 1: INTRODUCTION

RATIONALE

It is not a new phenomenon for criminals to hide or disguise evidence. Laundering facilities in the United States are easily available, which allow criminals to launder evidence easily. The American Council for Energy-Efficient Economy has classified the types of laundering access into five categories including: residential/ household use, coin-op laundries, multifamily laundries (college dormitories), on premise laundries (hospitals), and industrial laundries (laundry care businesses). In 2009, roughly 82% of households in the United States had clothes washers, which equates to roughly 93.2 million washer units for household use (Cluett, Amann, Chou, & Osann, 2013). This ease of access to laundering facilities as well as the relative privacy of the house allows for the possibility of laundering evidence, especially clothing.

The following three cases are examples of laundered clothing, which were a key piece of evidence linking individuals in a criminal trial, and demonstrate the variety of situations where analysis of laundered evidence may be probative.

Case 1: State Of Ohio V. Paul R. Davis, 1998

On April 15, 1995, Yvonne Goodson was discovered stabbed to death in the Erieview Motel in Lorain, Ohio. The same day the body was found, Paul Davis offered to pay for the use of a washing machine across the street, which was owned by Mrs. Rosario. During the investigation, Paul Davis had admitted to using the washing machine

to launder his bloody clothing. Police detectives went to Mrs. Rosario's house and found bloody clothing in and around the washer. When questioned, Mrs. Rosario recalled that when she was adding detergent to the washing machine earlier that day, she noticed that the water was "brick color." Blood found in the water samples from the washing machine hoses and on clothing that was nearby were all type 'O' that was consistent with the victim, Yvonne Goodson. DNA testing was performed and neither Ms. Goodson nor Mr. Davis could be excluded as a source of the mixed DNA profile associated with the washing machine (State of Ohio v. Paul R. Davis, 1998).

Case 2: State V. Pizzoferranto, 2005

In the early morning of February 18 2003, Officer Glenn Tucker was severely beaten outside Tommy T's bar in Stark County, Ohio. An informant told investigators that Nicholas Pizzoferranto had attempted to conceal evidence associated with the assault by washing his clothing and shoes. DNA profiling techniques used on the laundered clothing was used to identify the officer's blood (State v. Pizzoferranto, 2005).

Case 3: Grega V. Pallito, 2011

In 1994, Christine Grega was found dead with blunt force injuries in a rented condominium near Mt. Snow, VT. When investigators arrived, they found bloody clothing soaking in a washing machine. Multiple cuttings were taken from the clothing, and some DNA testing was done at the time, but the tests yielded inconclusive results. Nonetheless, Mr. Grega was found guilty of murdering his wife. Grega petitioned in 2011 and contended that DNA analysis had advanced since the time of the original testing in 1994. The samples from the washing machine should therefore be retested. The judge ruled that the samples should not be tested because the "biological material would be too

degraded to permit DNA reconstruction" and concluded that additional testing would be a waste of resources (Grega V. Pallito, 2011).

Even though there are cases with laundered evidence, criminal investigation laboratories do not have established protocol to examine these unique evidence sources. In addition, there are very few scientific research studies on laundered evidence with which to base an examination of evidence on.

The lack of information and standardized protocols could affect the possible significance and interpretation of laundered evidence. Due to the uncertainty, possible sources of information could be disregarded as in the case of Grega V. Pallito. It is important that the most appropriate techniques are used to analyze the evidence thoroughly. Not using all available evidence or treating evidence in an inappropriate manner could lead to an injustice.

BACKGROUND AND THEORETICAL FRAMEWORK

Forensic science is an amalgamation of different sciences and trades. Unlike other hard sciences such as chemistry or physics where a personal drive for knowledge is the motivation, forensic science grew out of necessity due to society's curiosity to understand what happened during a crime.

Using forensic science applications, professionals in the justice system investigate crimes. Techniques to analyze evidence must be developed and honed to suit the evidence available to prove beyond a reasonable doubt that a crime transpired. In order to establish a crime has occurred, three factors must be documented which are the victim, the location, and the perpetrator.

It is necessary to identify the perpetrator of a crime accurately so that the correct person is punished. In order to distinguish one person from another a unique set of characteristics for the individual must be established. Many methods were developed to determine the distinctiveness of perpetrators over the last few decades.

The Bertillon system was the first documented method to identify people and was developed by Alphonse Bertillon (Swanson, Chamelin, Territo, & Taylor, 2009). This technique used measurements of the human body paired with standardized photos to make a unique identification.

However, Bertillon's approach lost credibility after the case of William West and Will West, two people with very similar names and dimensions that were mistaken for each other while they served time in jail (Thornhill, 2011; Olsen, 1987).

Only two decades after Bertillon's technique was established, another method was developed called dactylography including the study of fingerprints. There is a

dispute regarding who first recognized that fingerprinting could be used to identify people. Despite this contention, Sir Edward Henry is credited with creating the system of classifying fingerprints that was popularized as an investigative tool in 1890s (Swanson, Chamelin, Territo, & Taylor, 2009). The first case to use fingerprints was a 1901 burglary where a dirty fingerprint was found on the freshly painted windowsill that was used to gain entrance to a house (Thompson & Black, 2007).

Unfortunately, even with the use of the Automated Fingerprint Identification System (AFIS), fingerprint comparisons are still ultimately performed by human technicians. Manual fingerprint analysis relies on the interpretation of the analyst and this process could lead to biases or lack of reliability. Where one expert might declare a match, another expert might arrive at a different or no conclusion.

During the early 1900s, it was discovered that human blood could be classified by its antibody type. This allowed certain subsections of the population to be excluded as a suspect based on what antigens they presented on the surface of their circulating blood cells. The nature of these tests reduced the role of a scientist's subjective opinion and potential for bias.

However, the discriminating power of ABO blood typing is relatively low. Several people in the US have been wrongly accused based on serology (Hampikin, West, & Akselrod, 2011) including Paul House who was convicted in 1986 of raping and murdering a woman in rural Luttrell, Tennessee. The verdict was partially based on ABO typing a bloodstain on clothing (Innocence Project, 2014). Mr. House was released in 2008 after serving 22 years in jail when new evidence identified the actual perpetrator of the crime for which he had been convicted (Innocence Project, 2014).

Although fingerprinting and serology helped tremendously in the fight against crime, they are susceptible to many faults and injustices. The uncertainty of these techniques left a need for a more scientific and less biased way of associating criminals with crime scenes.

In the 1980s, the study of the human genome led to the discovery of highly variable sections of Deoxyribonucleic acid (DNA) (Goodwin, Linacre, & Hadi, 2007). Most of human DNA is the same between people; however, there are 0.3-0.5% differences between individuals. These variations are called polymorphisms (Thompson & Black, 2007) and they do not affect the phenotype of the organism (Butter, 2012). The sections or positions of DNA that have polymorphisms are called locus (plural loci).

Also around this time, Sir Alec Jeffreys discovered that individuals could be identified by the characterization of the variable number tandem repeats (VNTR) (Jeffreys, Alllen, Hagelberg, & Sonnberg, 1992) they possessed at a number of loci scattered throughout the human genome. VNTRs are between 8 and 100 base pairs (bp) (Tautz, 1993) that repeat at least two times. When the loci are 'cut' and separated by size using gel electrophoresis, a pattern or 'barcode' is depicted which can be used to identify a person. Sir Jeffreys called his discovery "DNA fingerprinting" due to the physical appearance of the gels that were used to visualize test results (Taupin, 2014) (see Figure 1 & 2). However, the term 'DNA fingerprinting' was negatively viewed by those doing traditional fingerprinting and was misleading due to the randomness and inexact science of dactyloscopy. To reflect the differences in the forensic methods 'DNA profiling' or 'genotyping' terms were used to replace 'DNA fingerprinting'.

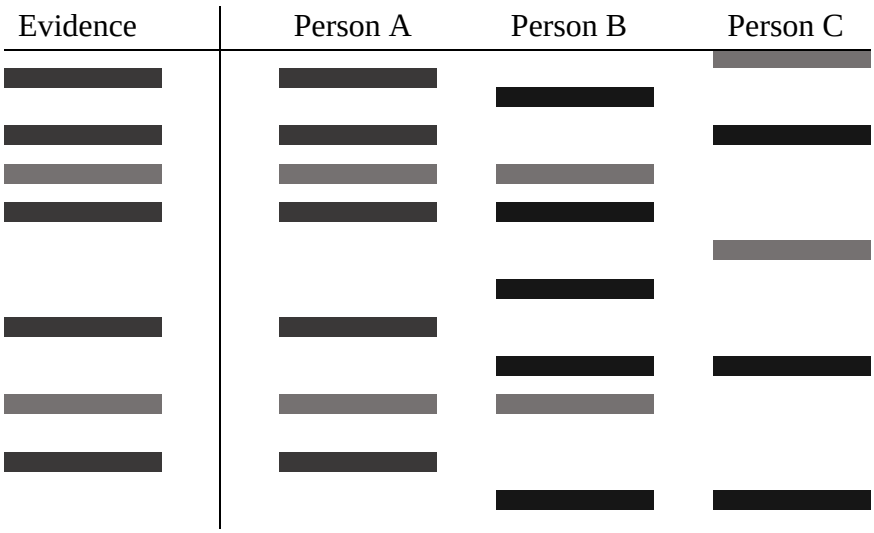

Figure 1: A cartoon example of DNA profiling. The gray and black lines indicate different lengths of fragmented DNA from larger to smaller fragments at the bottom. A comparison of the fragments in the 'Evidence' sample and 'Person A' is indicative of a ‘match’ since the lines are at the same distance away from the origin point (top horizontal line).

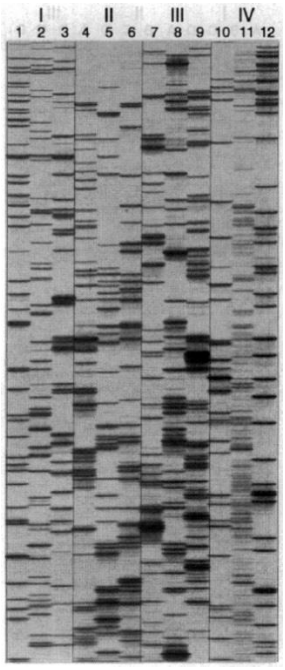

Figure 2: Example DNA profiling of amplified human and plant DNA using gel electrophoresis. Human DNA is depicted in colum IV, plant DNA is depicted in colums I (Arabidopsis), II (tomato) and III (maze) (Chial, 2015).

VNTRs are highly susceptible to degradation and require about a quarter sized droplet to get a profile. Due to these limitations scientists worked on a more reliable method of DNA analysis. This led to the discovery of microsatellites, or more commonly known as Short Tandem Repeats (STRs).

Current forensic DNA profiling techniques use STRs rather than other available techniques. This is due to STRs greater resistance to the effects of degradation, greater sensitivity, amenability to indexing, great discriminating power, and the possibility of getting test results in as little as a few hours.

STRs involve tandem repeats of between 2-7 bp. STR alleles derive their names from the number of times these repeats are present within a specific loci. Each person has two alleles at a locus with one allele being inherited from each parent. There is a possibility of getting the same allele in a locus from both parents. This situation would result in the individual being homozygous for that allele. If the alleles have a different number of repeats, the individual is heterozygous for that locus (see figure 2).

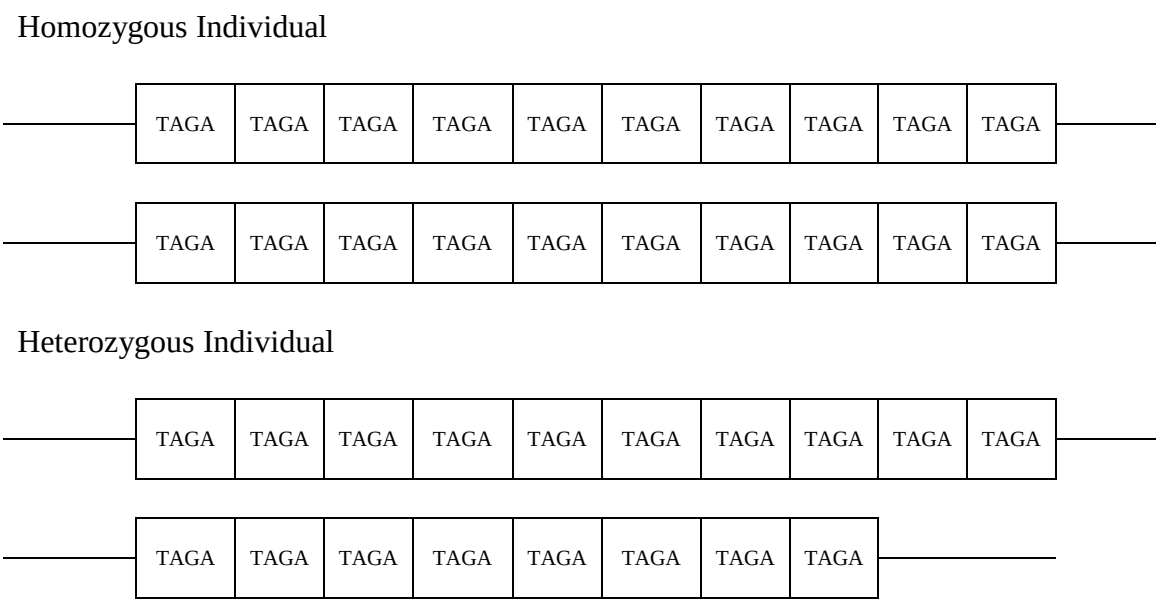

Figure 3: Simplified representation of Short Tandem Repeats (STRs) at one locus. Boxes indicate a base pair repeat with 'TAGA' being the repeating base pairs. In the homozygous individual 10 repeats were inherited from both parents. The heterozygous individual has one allele with 10 repeats and one allele with eight repeats, which were inherited from their parents.

STR polymorphisms have a small range of available alleles. This allows multiple loci to be tested at once to generate a profile called multiplexes. Manufacturers will make kits that look at different loci. Although, most kits include loci: FGA, vWA, D3S1179, D18S51, D21S11, THO1, and D16S539.

In modern DNA genotyping, a computer program can depict the STR polymorphisms by the horizontal position of a peak on a graph called an electropherogram. Using this method, multiple loci can easily be displayed on the same page to represent a person's STR DNA profile.

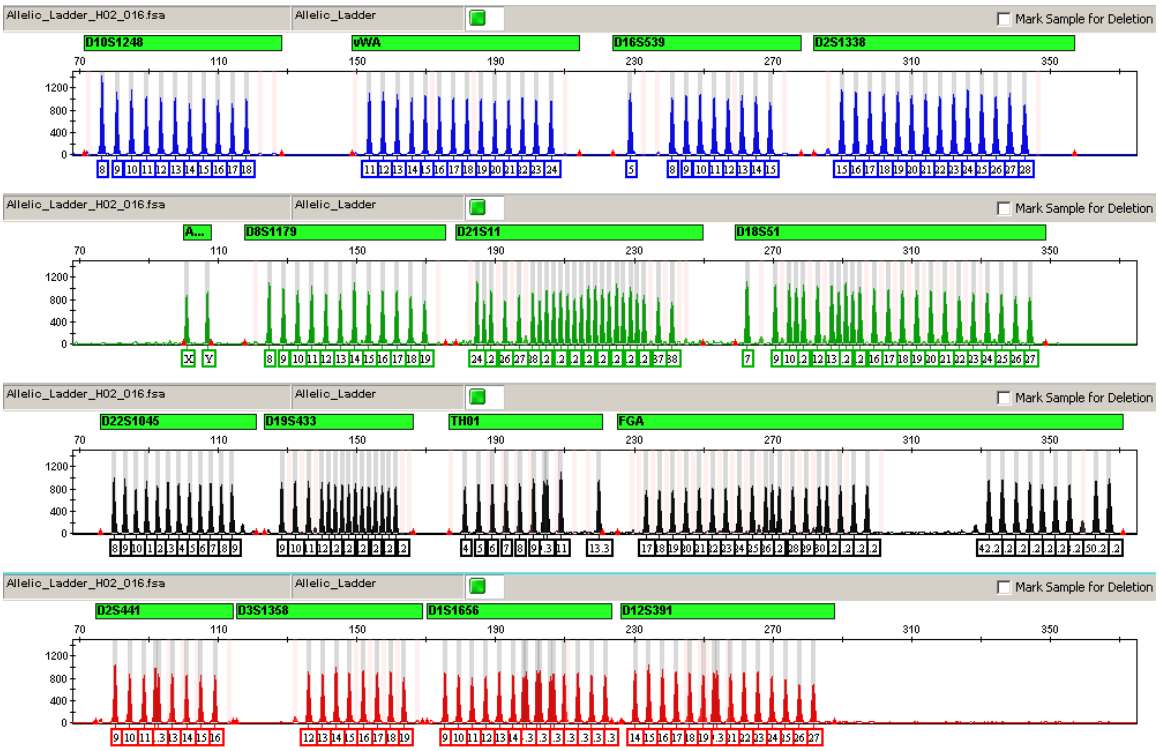

Figure 4: Electropherogram of an allelic ladder using GeneMapper ID-X Software for AmpFlSTR NGM. The peaks illustrate commonly observed alleles for 16 loci, represented by the green horizontal bars (Applied Biosystems, 2012).

In using STR analysis techniques, it only takes about 0.5 ng of DNA to get a DNA profile (Applied Biosystems, 2012). There are varying volumes of DNA that are stated as the minimum for profiling with the smallest amounts being called low-template DNA (LT-DNA) (Lawless, 2012). Some studies have even been able to get profiles from as little as 200 picograms (pg) or less (Van Oorschot, Ballantyne, & Mitchell, 2014; Aditya, Sharma, Bhattacharyya, & Chaudhuri, 2011; Meakin & Jamieson, 2013).

DNA has been the focus of forensics and research for the last few decades. DNA can be found in most nucleated cells (Butler, 2012; Thompson & Black, 2007) and can help investigators piece together what happened. Some common examples of DNA found

in crime scenes include cigarette butts, foods like apples cores, fingerprints, hairs, semen stains, fingernail clippings (Butter, 2012).

Although newer technology can analyze LT-DNA, scientists should be cautious since DNA is everywhere is our environment from one source or another. This low level of DNA on substrates is typically referred as background DNA. The smaller the amount of starting material the higher the likelihood that the background DNA will mask smaller samples (Gill, 2014).

Once DNA has been genotyped, the probability of determining the likelihood of another person having the same number of repeats is statistically calculated using the product rule (Butler, 2012). This is calculated by finding the frequency alleles, 2pq for heterozygotes and p^2 for homozygotes, and then multiplying the loci together. The product rule is then used on all of the loci detected and creates a multi-locus frequency in the quintillion values.

Once the frequency of a DNA profile is determined, two calculations evaluate the significance of a match. The first calculation indicates the possibility of finding a potential match to the same profile from a random set of unrelated individuals called random match probability (RMP). The RMP is used in cases of single source samples. The second calculation called combined probability of inclusion (CPI) shows the expected frequency of how many people could be a potential match. The CPI is used for DNA sources that are mixtures of at least two individuals.

When a DNA profile does not match that of a suspect, the unknown evidence sample profile can be loaded into the Combined DNA Index System (CODIS). CODIS is the FBI's computer database that allows law enforcement store all of the DNA profiles

collected in the investigation and conviction of a felony crime. This program allows unsolved cases to be linked together so that cold cases can be solved by pairing unknown DNA profiles with previous cases.

DNA cannot only help convict offenders but as of August 2013, DNA has exonerated the names of 343 post-convicted people who were wrongly convicted of serious crimes (Innocence Project, 2016). Of those 343 people, eighteen innocent people served time on death row (Innocence Project, 2016).

One of the most notable turning points in public knowledge and acceptance of DNA evidence was the heavily publicized trial of O.J. Simpson in the early 1990's. This trial sparked the public interest in the justice system, which the entertainment industry was happy to meet with the production of crime solving shows like 'CSI: Crime Scene Investigation'. The fast-paced plots, and ease of evidence analysis in these shows has raised the public's level of familiarity with forensic sciences (Harriss, 2011).

Public awareness of forensic science techniques can help the jurors understand evidence better, but can also hinder the process as juries are now expecting DNA evidence in every case. As a result, juries are making convictions harder to secure in court due to a 'lack of evidence,' colloquially known as "the CSI effect" (Harriss, 2011).

A prosecution-friendly side to the CSI effect and television crime shows tends to put laboratory personnel or experts on a pedestal in the eyes of the jury. Due to the sway of an 'expert', it is necessary to correctly evaluate and represent evidence fairly in court.

With limitless funding and time, all evidence should be thoroughly investigated; however this is impractical for every case. Justice professionals are forced to send only

the most viable and valuable samples for analysis. Unfortunately, there are limited studies to scientifically direct these choices.

Identification of individuals has progressed rapidly in the last century and especially with the discovery of polymorphisms in the last century. Documentation shows that criminals have hidden DNA evidence and their involvement in crimes. A possible source of DNA evidence is laundered clothing and could link perpetrators to criminal events. As a result, forensic scientists should focus their attention on the possible source of identification from laundered clothing.

STATEMENT OF THE PROBLEM

Laundered DNA evidence can easily be found at crime scenes and could exclude or include suspects in a crime. Most evidence is either treated without special considerations for the effects of laundering will have on DNA recovery or ignored entirely.

Previous research has examined one factor, such as one type of detergent (Cox, 1990, Castello et al., 2009, 2010, 2012) and exaggerated its effects to test the ability to collect useful information from the evidence. Additionally, the transfer of spermatozoa (Kafarowski, Lyon, & Sloan, 1996) and epithelial cells (Petricevic, Bright, & Cockerton, 2006) in the washing machine has shown to occur in a few research studies. Unfortunately, these types of studies are very limited, and do not take into account the hundreds of combinations of variables for laundering (see Figure 4).

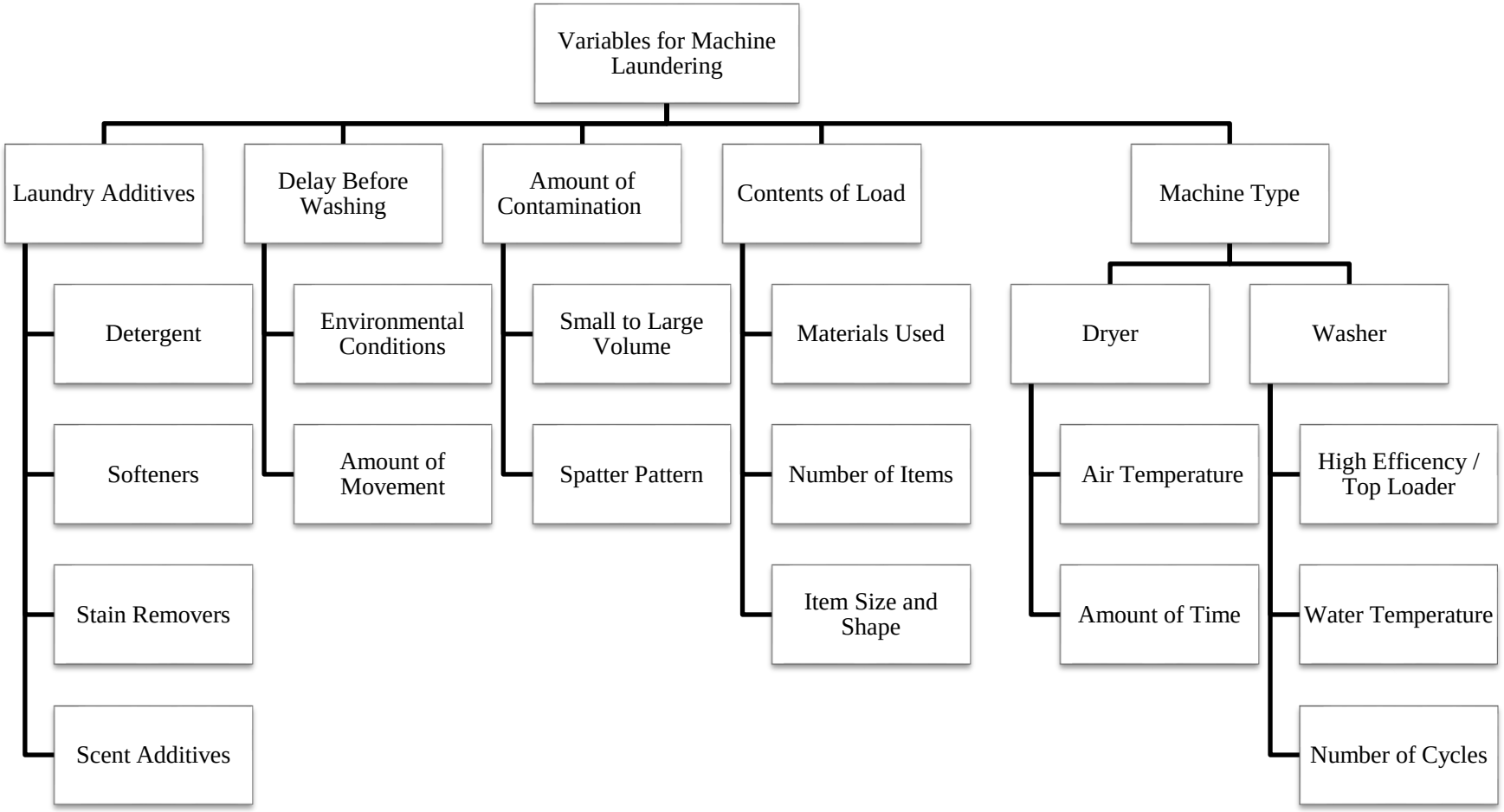

Figure 5: Possible variables affecting DNA recovery in machine laundering.

Without sufficient scientific studies to support decisions, investigators are unwilling to send out samples, lab personnel are unsure on how best to treat the material, and judges are unwilling to accept the evidence in court (Grega V. Pallito, 2011).

Failure to recognize evidence might lead to innocent people being convicted, leaving organizations like the Innocence Project to test samples and petition for retrials (Hampikin, West, & Akselrod, 2011). This process is time consuming and costly for the defendant as well as the public and leaves an open need to study DNA on laundered clothing.

Five key areas if studied should give important insights into most commonly thought of scenarios for laundering to effect DNA evidence. These are: 1) the effects of different laundry additives, 2) time between deposit and washing, 3) amount of contamination, 4) contents of load, and 5) machine type.

With the consideration of the five key areas, and the need to make a foundation for further research, this study focuses on collecting and analyzing DNA profiles from simulated laundry loads and addresses the following four research questions:

1. What are the effects of laundry additives on the amount of recoverable DNA for a DNA profile?
2. What is the effect of a delay between deposit and laundering on recovering a DNA profile?
3. Will the amount of blood deposited affect the amount of DNA recovered?
4. During laundering, can DNA be transferred from one garment to another garment in a primary load or between a primary to a secondary load or a tertiary load?

Modern Clothing can be constructed from a wide range of materials with infinite combinations. Laundering machines can be broadly be categorized, however they have an exceedingly large combinations of options with temperature, duration, brand, mechanism method etc. Due to internal complications of clothing materials and type of washing machines it would be prudent to study these factors separately.

SUMMARY

For all techniques used in forensic science there is a continual need for adaption to new situations and new scenarios to criminal cases to make sure justice is served. DNA analysis has changed over time and has become a useful tool in the field of forensics, nonetheless, it is still subject to being developed. Even though there are multiple cases each year that involve laundered evidence, the forensic scientific community has yet to address the possible value of laundered DNA evidence.

Laundry is a part of our everyday lives and criminals can use this everyday tool to their perceived advantage to hinder the recovery of DNA. The prejudiced notions in the minds justice professionals about laundering machines have hindered the advancement in the interpretation of laundered evidence. This study has investigated four research questions of laundering in the attempt to educate the possibilities of laundered DNA

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CHAPTER 2: RECOVERABILITY OF DNA AFTER EXPOSURE TO LAUNDRY ADDITIVES

ABSTRACT

Criminals use the chemical properties of additives to attempt to remove DNA laden material from clothing. This research looked at the effects of 18 different laundry additives on the amount of recoverable DNA for a DNA profile in three parts. Part one looked at an equal ratio of human blood to laundry detergent. Part two then looked at a diluted ratio of laundry additives on human blood. Finally, Part three looked at the most and least effective laundry additives on a bloody shirt during machine laundering. The results of this study suggest that even with the most effective laundry additive, a full DNA profile is recoverable even after being machine-washed. Contrary to popular belief, the data also demonstrates that laundry additives are more effective at a diluted ratio then at an undiluted one. These results suggest that DNA that is exposed to laundry additives should still be tested for evidentiary value.

INTRODUCTION

Most people understand how to use laundering additives. More importantly, many know that these additives remove stains. Laundering additives include anything used for laundering purposes such as detergents, softeners, scent boosters, and bleaches. In developed countries, laundry additives are easily available for purchase. In addition, consumers can select from a wide range of options in manufacturers and processing aids (such as fragrances). Due to their one time use, laundry additives have a high rate of consumption. In 1998, it was estimated that 3,660,000 tons of laundry detergents and 1,000,000 tons of fabric softeners were used annually in Europe alone (Bajpai & Tyagi, 2007).

Laundering clothing is simple and mostly automated using modern equipment. Because of the ease of laundering, it is possible that criminals capitalize on this and attempt to hide their involvement in a crime by laundering their evidence-laden clothing. Even though laundered clothing is a possible source of evidence, the forensic community has limited studies (Castello, Frances, & Verdu, 2012; Castello, Frances, & Verdu, 2010; Castello, Frances, & Corella, 2009; Cox, 1990) on effectiveness of laundry additives to hinder the recovery of DNA from clothing.

The first laundry detergents made out of synthetic surfactants began during a shortage of fat to make soap in World War II (Bajpai & Tyagi, 2007). Modern laundry additives now include a variety of ingredients to increase laundering machine effectiveness and reduce deterioration of the machine itself. Each ingredient can fall into the following categories: surfactants (chemical removal of stains), builders (makes surfactants more efficient), anti-redisposition agents, zeolite (keeps surfactants in

solution), alkaline agents, corrosion inhibitor, processing aids, colorants, fragrances, oxygen bleach, suds control agents, opacifiers, bleaching agents, enzymes, and specializing components (Bajpai & Tyagi, 2007). The combination of ingredients gives laundry additives a unique appearance and determines stain specificity, increasing consumer appeal.

Currently, crime laboratories do not have a universal procedure to anticipate potential interactions between detergents and extraction methods. This institutional negligence is especially troubling because detergents' intended purpose is to remove stains. Some experiments were completed to address the effectiveness of laundry additives but none has attempted a full-scale test representative of evidence found at a crime scene.

Crime investigators use preliminary tests to locate DNA material to prioritize the collection of stains at crime scenes. The stain will not be collected for further analysis if the test is negative. Publicly available laundry additives contain different chemicals at different proportions that may affect the tests used for preliminary testing.

One of the concerns for preliminary testing was sodium percarbonate (more commonly known as active oxygen), which was introduced as a laundry additive in the early 1990's. Between 2009 and 2012, three experiments were conducted on the effectiveness of sodium percarbonate, on the detection of evidence and subsequent DNA analysis.

In two of the experiments, Castello et al. placed five drops of blood on 100% cotton fabric samples and soaked the fabric in active oxygen containing solutions for two hours. Preliminary testing with phenolphthalein, reduced phenolphthalein, Luminol,

Bluestar, and human hemoglobin (Hexagon OBTI) rendered negative results even though the stain was still visible (Castello, Frances, & Corella, 2009; Castello, Frances, & Verdu, 2012). In the third study, it was found that a DNA profile could be obtained even from the stains that presented a negative preliminary result (Castello, Frances, & Verdu, 2010).

This research demonstrated that human DNA suitable for genotyping could be obtained from bloodstains even after they were exposed to sodium percarbonate. Thus far, current research has failed to directly address three central phenomena: 1) garment movement within a washer; 2) water cycle options and characteristics; and 3) the possibility that DNA might be transferred between garments.

In 2012, a study of the effects of diluted detergent on nuclear DNA in zebrafish *Danio rerio* (Cyprinidae) showed that 24% of exposed fish experienced DNA strand break damage (Sobrino-Figuero, 2013). The fish were subjected to 16 days of water treated with detergent at the same concentrations found in waterways. The water samples were collected from water sources, which were contaminated by untreated wastewater from residential areas. It was hypothesized that this DNA deterioration resulted from surfactants found in laundry detergents (Sobrino-Figuero, 2013).

This research demonstrated that diluted detergents could damage DNA when exposed to the solution for long periods. Due to human social conventions and directions in washing machine manuals (Kenmore, 1999), some people will soak laundry before washing. Many washing machines can even be programed to soak laundry before washing. However, it would not be expected that a criminal would soak evidence-laden clothing for 16 or more days to replicate the slow rate of decay of DNA strands as seen in the fish cells.

In direct opposition to laundry detergents degrading DNA, there are several experiments done using regular laundry detergent as a scientific method to extract DNA from cells. Phenol and other harsh chemicals are used traditionally to lyse and extract DNA from cells (McClintock, 2008), even though these chemicals are toxic and pose a threat to researchers (Acros Organics, 2009). Some laundry detergents have ingredients that can extract the DNA in a similar manner to traditional systems and have no significant reduction in material for non-forensic casework (Nasri, Forouzandeh, & Rasaee, 2005). Despite that fact, it is unclear if using laundry detergent before using a traditional extraction method such as phenol-chloroform isoamyl alcohol would degrade the DNA material too much to generate a profile.

In order for a soil to be removed from the substrate, the energy of the soil must increase from the low energy level (stationary on the substrate) to a higher energy level (being in solution) (Bajpai & Tyagi, 2007). Laundry additives lower the activation energy so that the soil can easily be removed from the substrate.

The change in activation energy of removing using detergents has been overlooked. As previous studies have only soaked bloodstains in the detergent solutions (Castello, Frances, & Verdu, 2012; Castello, Frances, & Verdu, 2010; Castello, Frances, & Corella, 2009) or tested the washing mechanism without detergent (Kafarowski, Lyon, & Sloan, 1996).

Due to the limited amount of additives tested, limited research of the combination of laundry additives and movement within laundering, and conflicting results of whether or not detergents effect the quality of DNA, laundered evidence is treated with some ambiguity. The doubt over the quality of the DNA has caused it to be ignored as a

possible source of evidence in criminal investigations. The lack of information and the existence of laundered evidence suggest a need for the study of the effects of laundry additives on DNA evidence laden clothing.

To address the direct effects of laundering additives a wide range of available solutions should be directly applied to blood to see if the chemical interactions would affect forensic DNA analysis.

To see the effects of the movement in the washer with laundry additives a full-scale test should be used to see if the physical and chemical processes would be enough to hinder the recovery of DNA. A control of a laundered shirt without laundering additives would allow for the comparison of the combined physical and chemical effectiveness of the additives. As blood is the most visible and frequently found biological fluid found in crime scenes, it would be logical to use blood as a source of DNA for this study.

Taking into consideration the findings of this literature review, the research presented here addressed the question of ‘what are the effects of laundering additives on the amount of recoverable DNA for a DNA profile’ with the following hypotheses:

1. Exposing blood to undiluted laundry additives will have no effect on the recovery rate of DNA.
2. Exposing blood to diluted laundry additives will have no effect on the recovery rate of DNA.
3. Subjecting blood to machine laundering with laundry additives will have no effect of the recovery rate of DNA.

This knowledge may help limit production or sale of responsible chemicals that would hinder the recovery of DNA in criminal investigations. Multiple chemicals have been removed from laundry additives over the years due to their effects on the environment or other factors (Bajpai & Tyagi, 2007). It could be suggested that another ingredient that would not affect the DNA be substituted for a chemical that hinder criminal investigations.

This research may help investigators reconsider the evidentiary value of samples collected from crime scenes. As a result, it could help solve cold cases. It may also lead to further study that helps investigators understand the process of the degradation of laundry additives on DNA. Additionally, in follow up studies it may possible to reduce the effects of degradation on DNA material if possible evidence is found in the process of laundering.

METHODS

To test the effectiveness of laundry additives on removing or denaturing DNA, the study was split into three parts. Parts one and two were used to examine the direct interaction of laundry additives on human blood at undiluted and diluted strengths. The undiluted strength was used to simulate the direct contact or application of a laundry additive on a bloodstain. The diluted strength was to simulate the concentrations found in the washing machine if the recommended amount was used to launder a bloodstain. Part three was used to simulate a possible laundering of a bloody t-shirt with the most and least effective detergents in a full scale test with a top loading washer.

Blood used for this study was obtained from the Community Blood Center/Community Tissue Services (CBC/CTS) in Dayton, Ohio. The blood was donated by volunteers in the Dayton, Ohio area. All donors were given written notice, and consented to the use of their blood in unspecified research. No demographics were released to the researcher at any time, and the researcher did not have any contact with the donors. A Genotype profile was generated for comparison of samples collected (see Table 1).

Table 1: Genotype profile for Subject 1

Loci	Subject 1
D10S1248	13, 16
vWA	17, 18
D16S539	11, 12
D2S1338	25, 26
Amel	X, X
D8S1179	12, 13
D21S11	29, 31
D18S51	13, 15
D22S1045	16, 16
D19S433	14, 15
TH01	9, 9.3
FGA	18, 25
D2S441	10, 14
D3S1358	17, 18
D1S1656	14, 18.3
D12S391	24, 24

Part One: Undiluted Laundry Additives Combined with Blood

Eighteen laundry additives were used for this study and were classified into nine laundry additive categories such as stain remover (see Table 2) based on the additive's contents (see Table 23 in Appendix A) and labeling. One ml of laundry additive one was combined in a 2 ml test tube with 1 ml of Subject 1's blood and inverted five times to mix. This procedure was repeated for all 18 laundry additives each with two experimental replicates and three technical replicates (N=108). Powdered additives were combined in a weigh boat and stirred with a toothpick until a consistent texture was achieved. A negative control was made with 2 ml of autoclaved water. A positive control was made with 1 ml of autoclaved water and 1 ml of Subject 1's blood. A sample (100 µl) of each combination was transferred into separate 1.5 ml test tubes for extraction.

Table 2: List laundry additives used for the study.

Additive Category	Manufacturers Label
Hypoallergenic	all® 2X Ultra Free Clear Laundry Detergent, 50 oz.
High Efficiency	all® HE 2x Ultra Oxi-Active® Stainlifters™ Laundry Detergent, Waterfall Clean, 50 oz.
Bleach	Ajax® 2x Ultra Liquid Laundry Detergent with Bleach Alternative, 50 oz.
Softener	Downy® Non Concentrated Fabric Softener, April Fresh, 64 oz.
Concentrated	Ultra Gain® 2x Liquid Laundry Detergent, Original , 50 oz.
Active Oxygen	Clorox® Green Works™ Oxi Stain Remover, Unscented, 56 oz. Container
High Efficiency	Method® HE Laundry Detergent, Free And Clear, 20 oz.
Natural Ingredients	Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.
Hypoallergenic	Tide® Free & Gentle 2x Concentrated Laundry Detergent, 100 fl. Oz
Bleach	Tide® Powder Laundry Detergent with Bleach, 144 oz.
High Efficiency	Tide® HE Laundry Detergent, Original Scent, 100 oz.
Packets of Detergent	Tide® PODS™ Spring Meadow, 72 Ct.
Concentrated	Wisk® 2X Liquid Detergent, Deep Clean, 50 fl oz
Softener	Bounce® Fabric Softener Sheet, Outdoor Fresh
Stain Remover	Tide® To Go Pen
Active Oxygen	OxiClean® Max Force® Laundry Stain Remover Spray, 12 oz.
High Efficiency	Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, 50 oz.
Hypoallergenic	Seventh Generation® Free & Clear Natural Laundry Detergent, Unscented, 112 oz. Box

Part Two: Diluted Laundry Additives Combined with Blood

Each detergent was diluted with autoclaved water to the percentage found in a medium sized load for a washer (see Table 3). To calculate the detergent percentage found in a medium-sized load the following equation was used:

$$Detergent\ Percentage\ in\ a\ Medium\ Sized\ Load = \frac{Manufacturers\ Recomended\ Volume\ per\ Load\ (ml)}{69887.5\ ml}$$

The recommended amount of laundry additive per load (see Table 3) listed on the product container, was used during the experiments. When the manufacturer did not recommend a volume, 1 ml of the solution was used. On average, the washer used 69.88 L of water in a medium sized load (see Table 25 in the Appendix A).

Table 3: Percentage of laundry additive found in a medium sized load of laundry based on the manufacturer's recommended volume.

Laundry Additive Label.	Manufacturer Recommended Volume Per Load	Detergent % In 'Medium' Sized Load In Washer
all® 2X Ultra Free Clear Laundry Detergent, 50 oz.	44.36 ml	0.063
all® HE 2x Ultra Oxi-Active® Stainlifters™ Laundry Detergent, Waterfall Clean, 50 oz.	52.94 ml	0.076
Ajax® 2x Ultra Liquid Laundry Detergent with Bleach Alternative, 50 oz.	46.13 ml	0.066
Downy® Non Concentrated Fabric Softener, April Fresh, 64 oz.	90.20 ml	0.129
Ultra Gain® 2x Liquid Laundry Detergent, Original , 50 oz.	46.13 ml	0.066
Clorox® Green Works™ Oxi Stain Remover, Unscented, 56 oz. Container	39.00 ml	0.056
Method® HE Laundry Detergent, Free And Clear, 20 oz.	11.83 ml	0.017
Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.	44.66 ml	0.064
Tide® Free & Gentle 2x Concentrated Laundry Detergent, 100 fl. Oz	46.13 ml	0.066
Tide® Powder Laundry Detergent with Bleach, 144 oz.	53.23 ml	0.076
Tide® HE Laundry Detergent, Original Scent, 100 oz.	46.13 ml	0.066
Tide® PODS™ Spring Meadow, 72 Ct.	23.66 ml	0.033
Wisk® 2X Liquid Detergent, Deep Clean, 50 fl oz	46.13 ml	0.066
Bounce® Fabric Softener Sheet, Outdoor Fresh	146.30 cm^2	N/A
Tide® To Go Pen	Not stated	0.001
OxiClean® Max Force® Laundry Stain Remover Spray, 12 oz.	Not stated	0.001
Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, 50 oz.	46.13 ml	0.066
Seventh Generation® Free & Clear Natural Laundry Detergent, Unscented, 112 oz. Box	47.32 ml	0.068

White 100% cotton cloth was used as a substrate for samples. Cloth was sourced from a local fabric store. The fabric was cut into 5 cm^2 pieces on lab counter sheeting using gloves and autoclaved scissors. Fabric samples were placed on a piece of aluminum foil that was at least 11x6 cm. The aluminum was then folded over to cover the fabric. After that, the three open sides were folded twice to enclose the fabric. A piece of autoclave tape was then placed on the outside of the packet (see Figure 6). The wrapped fabric was autoclaved to 120^oC for 20 minutes and then allowed to cool to room temperature. The fabric was stored in the foil until use and was opened in a DNA reduced environment in a biological safety cabinet.

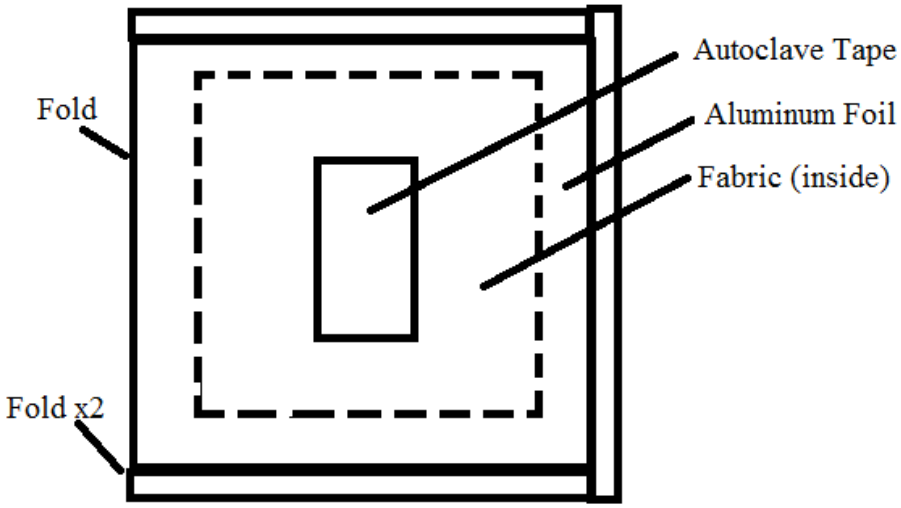

Figure 6: Diagram of fabric substrate preparation. A 5 cm^2 piece of fabric was folded into an aluminum pouch in preparation for autoclaving.

Once the Laundry additives were diluted and the fabric was ready, 1 ml of each diluted laundry additive, 1 ml of subject 1's blood, and one 1 cm^2 piece of fabric was combined in 2 ml test tubes and inverted 5 times to mix. This procedure was repeated for all 18 laundry additives with three experimental replicates (N=54). A negative control

was made with 2 ml of autoclaved water. A positive control was made with 1 ml of water and 1 ml of Subject 1's blood. The fabric of each combination was transferred into separate 1.5 ml test tubes for extraction.

Part Three: Machine Laundering With The Most And Least Effective Additives And Detergents.

A medium sized T-shirt (Gildan 5000) was used as the substrate for the samples. To prepare the T-shirts, a piece of autoclave tape was folded in with each item inside an autoclave bag, and a short piece of autoclave tape was placed on the outside of the bag. The bags were autoclaved at 120^{o}C for 20 minutes and then allowed to come to room temperature. The fabric was stored in the bag until use and was opened in a DNA reduced environment within a biological safety cabinet. If the autoclave tape that was folded with the clothing was not showing a positive reaction for sterilization, then the clothing was not used. The prepared fabric or shirts were spread out on a clean sheet of bench paper with the plastic side up in a clear plastic container (see Figure 7) inside a Biological Safety Cabinet (BSC).

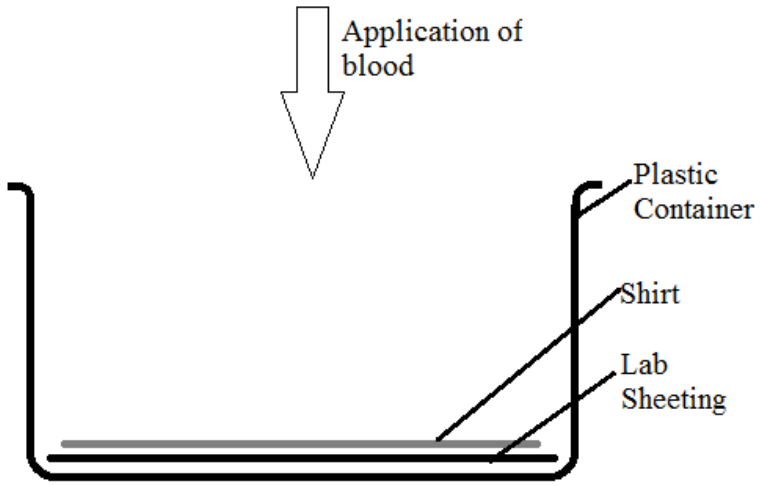

Figure 7: Diagram of application of blood onto a prepared shirt

The blood was transferred from the donor bag into an autoclaved glass container and stirred. The blood was applied from a height of 10 cm. 10 ml of Subject 1's blood was applied to a prepared T-shirt and washed immediately with the recommended amount of laundry additive '4' on a 'medium' water level with a 'warm, warm' water temperature on the 'regular' speed setting for 10 minutes. The shirt was transferred to the dryer set to 'normal/perm press' temperature for 50 minutes.

This was repeated with laundry additives 7 and 8 each with three replicates (N=9). The machines used were a 2003 top loading Kenmore (model number 20712990) washer and Kenmore (model number 110.60902990) dryer. The machines are located at Wright State University and would be classified as an onsite laundering facility (Bajpai & Tyagi, 2007). This room has one exterior door and keypad locked interior entrances and is accessible to professionals at Wright State University (see Figure 15in Appendix A). The machines are typically used to clean lab coats or other cloth materials used on campus.

Before use and between each load, the machines were sanitized using 20% bleach solutions and allowed to stand for 20 minutes. Fabric cuttings (1 cm^2) were cut from the center of the shirts and stored in a 1.5 ml test tube for extraction. The cuttings were taken using a mortise bit (see Figure 8) to reduce variability of sample size. The rest of the shirt was stored in labeled paper evidence bags.

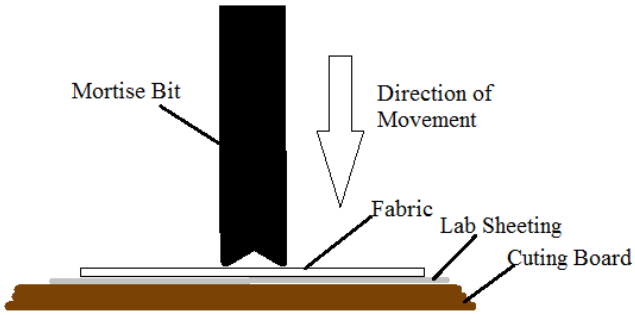

Figure 8: Diagram showing the cutting of fabric samples

Extraction

This procedure was adapted from AmpFlSTR Profiler Plus PCR Amplification Kit User's Manual (Applied Biosystems, 2012) and Forensic DNA Analysis: A Laboratory Manual (McClintock, 2008). All samples collected for extraction were combined with 500µl of digest buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, pH 7.5) and 15 µl of 10 mg/ ml Proteinase K (Applied Biosystems, 2012). The solution was vortexed for 15 seconds and incubated between eight hours at 56 ^oC. Phenol-Chloroform Isoamyl Alcohol (PCIA) (500µl) was added to each tube and vortexed for 15 seconds and centrifuged at 1500 rpm for five minutes. The aqueous layer

then was transferred to a new test tube and the PICA step was repeated two more times. Ethanol (1 ml) was added to each tube and incubated for 30 minutes at 0 ºC. The samples were centrifuged for 15 min at 1500 rpm and the liquids removed. Ethanol (1 ml) was added to each tube and vortexed for 15 seconds. The samples were centrifuged for 15 min at 1500 rpm and the liquids removed. The test tubes were allowed to air dry inside of a biological safety cabinet. 36µl of 1x TE-Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) was added to each sample and stored at 0 ºC (McClintock, 2008).

Quantification

Extracted DNA was quantified using the standard protocol from Applied Biosystems for the Human Quantifier Kit User Manual (Applied Biosystems, 2012). ABI Prism® 7900HT Sequence Detection System (Applied Biosystems, 2001) was used to collect data. Either sample (2 µl) or standard (2 µl) was added to separate wells in a MicroAmp Optical 96-Well Reaction Plate (Life Technologies). Master Mix (23 µl) (10.5 µl Quantifier Human Primer mix, 12.5 µl Quantifier PCR Reaction mix) was added to each well and the plate sealed with optical adhesive cover. Thermal cycling parameters consisted of stage one at 95 ºC for 10min, stage two was 95 ºC for 15 seconds then 60 ºC for 1 minute with 40 ºC repeats. The IPC and Quantifier Human detectors were set with a standard curve and the 9600 emulation option on.

DNA standard series were made using a stepwise dilution of Quantifiler™ Human DNA Standard with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0 (McClintock, 2008)) following the manufacturer's recommendations. A series of eight standards were run in duplicate with the concentrations ranging from 0.023 ng/µl to 50 ng/µl.

STR Amplification

Using the quantification data, 10 ng of the samples were transferred and air-dried in PCR tubes. The PCR tubes were then shipped to the University College of London in London, England. Polymerase Chain reaction (PCR) was conducted on DNA extracts using AmpFlSTR® NGM™ PCR Amplification Kit (Applied Biosystems, 2012). Samples were suspended to a concentration of 1 ng/µl, with 1µl of each sample added to separate PCR test tubes with 15 µl of Master Mix (10.0 µl AmpFlSTR NGM Master Mix, 5.0 µl AmpFlSTR NGM Primer Set). Thermal cycling parameters consisted of 1 minute at 95 ºC, followed by 29 cycles of 20 seconds of denaturation at 95 ºC and 3 minutes of annealing at 59 ºC with a final extension for 10 minutes at 60 ºC.

STR Capillary Electrophoresis

Standard or amplified DNA (10 µl) was added to separate wells on a 384 well plate and centrifuged of 5 seconds at 1500 rpm. Samples were analyzed on the Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, 2010). The run parameters consisted for 5-second injection period, 3 kV per 5 seconds injection voltage, 15.0 kV run voltage, for 120 min, as a 'standard run', with a capillary length of 50cm.

Data A**nalysis**

Quantification Data from the 7900HT Sequence Detection System was analyzed using Statistical Package for Social Sciences® Version 21.0 (IBM, 2012). Two ANOVA analyses were run on the data with two types of post-hoc analysis to account for the type of data collected.

To control for the type one errors an ANOVA with a Dunnett's Test was used a many-to-one comparison to test if anyone given laundry detergent with blood was different then blood with water. This can be calculated by the equation

$$q = \frac{Control\ mean - The\ mean\ of\ the\ test\ variable\ set}{Standard\ Error\ of\ Difference}$$

For all comparisons, an overall alpha = 0.05 level of significance was used to determine if the data sets were significantly different. To account for the assumptions of normality and constant variance needed for a Dunnett's Test, the square root of each datum on the amount of DNA recovered were used for the comparison.

To test if there was a significant difference between undiluted results and diluted results for each laundry additive, an ANOVA test with a Bonferroni correction to control for the inflated error rate when doing multiple comparisons was used. This correction yielded an alpha = 0.0013 level of significance.

Capillary electrophoresis data was analyzed using GeneMapper version 4.0 (Applied Biosystems, 2006). Recorded alleles exceeded a minimum threshold of 50 relative florescence units (RFUs). If a minor profile was observed then the minor profile was identified by using the assumption that certain alleles were indicative of the original planned profile and had normal morphology for alleles rather than artifacts.

RESULTS

The average recovery of DNA from all the undiluted laundry additives was 16.7 ng/µl ± 4.58 S.E.M., which is 13.2% of the total amount of blood projected in the original sample (Table 4). With the exception of 'all HE 2x Ultra Oxi-Active® Stainlifters™ Laundry Detergent, Waterfall Clean'($Q = 1.369$, $p>0.05$), and 'Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.' ($Q= 1.543$, $p>0.05$), all laundry additives resulted in a lower recovery rate than the positive control (blood combined with water) ($p<0.05$, Table 4).

The average recovery of DNA from all the diluted laundry additives was 2.68 ng/µl ± 1.56 S.E.M., which is 2.2% of the total amount of blood projected in the original samples (see Table 4). When comparing q-values, there was a significant difference between all of the means for the diluted laundry additives and the mean for the positive control (blood combined with water) ($p<0.05$, Table 4).

Thirteen out of the eighteen laundry additives had a reduction in the amount of DNA recovered from diluted laundry additive use relative to undiluted. Only laundry additives 'all® HE 2x Ultra Oxi-Active®' ($p = 0.0008$), 'Ajax® with Bleach Alternative' ($p = 0.0013$), and 'OxiClean®' ($p = 0.0013$) had significantly different recovery values between the undiluted and diluted samples (see Table 4).

STR profiles for samples exposed to either undiluted or diluted laundry additives were consistent with the expected profile of Subject 1 (see Table 5 &Table 6, for electropherograms see Figure 16 to Figure 17 in Appendix B). STR profiling of blood with undiluted Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, had

abnormal peaks and allele dropout. However, identified peaks in 6 out of the 16 loci used were consistent with subject 1's profile.

Table 4: Quantity of DNA (ng/µl) recovered from blood exposed to undiluted and diluted laundry additives. [a] Value significantly different then the blood baseline data. [b] Values for diluted and undiluted for the laundry additive is significantly different.

Laundry Additive	Experimental Replicate	1 ml of Laundry Additive to 1 ml of Blood			% Recovered	1 ml Diluted Laundry Additive to 1 ml of Blood			% Recovered
		Technical Replicate (ng/µl)				Technical Replicate(ng/µl)			
		1	2	3		1	2	3	
Blood Baseline	1	114.03	108.6	134.6		114.37	-	-	
	2	53.467	169.87	174.22		138.1	-	-	
	3	-	-	-		108.9	-	-	
	Mean ± S.E.M	125.80	±	18.29	-	120.45	±	8.96	-
all® 2X Ultra Free Clear Laundry Detergent [b]	1	0.00	0.00	0.00		2.71			
	2	28.47	20.39	15.08		12.30			
	3	-	-	-		4.04			
	Mean ± S.E.M	10.66 [a]	±	5.07	8.47	6.35 [a]	±	3.00	5.27
all® HE 2x Ultra Oxi-Active® Stainlifters™ Laundry Detergent, Waterfall Clean	1	80.676	76.748	32.149		5.48			
	2	82.55	40.87	40.36		4.43			
	3	-	-	-		3.53			
	Mean ± S.E.M	58.89	±	9.55	46.81	4.48 [a]	±	0.56	3.72
Ajax® 2x Ultra Liquid Laundry Detergent with Bleach Alternative [b]	1	26.264	25.777	24.149		0.11			
	2	25.36	23.05	17.60		0.00			
	3	-	-	-		0.12			
	Mean ± S.E.M	23.70 [a]	±	1.31	18.84	0.08 [a]	±	0.04	0.06
Downy® Non Concentrated Fabric Softener, April Fresh	1	0.04	0.02	0.01		0.07			
	2	0.01	0.00	0.00		0.07			
	3	-	-	-		0.07			
	Mean ± S.E.M	0.01 [a]	±	0.01	0.01	0.07 [a]	±	0.00	0.06

Laundry Additive	Experimental Replicate	1 ml of Laundry Additive to 1 ml of Blood			% Recovered	1 ml Diluted Laundry Additive to 1 ml of Blood			% Recovered
		Technical Replicate (ng/µl)				Technical Replicate(ng/µl)			
		1	2	3		1	2	3	
Ultra Gain® 2x Liquid Laundry Detergent, Original	1	0.00	0.00	0.00		0.01			
	2	35.96	2.92	0.00		0.00			
	3	-	-	-		0.52			
	Mean ± S.E.M	6.48 [a]	±	5.92	5.15	0.18 [a]	±	0.17	0.15
Clorox® Green Works™ Oxi Stain Remover, Unscented	1	0.00	0.00	0.00		0.39			
	2	0.00	0.00	0.00		0.39			
	3	-	-	-		0.63			
	Mean ± S.E.M	0.00	±	0.00	0.00	0.47 [a]	±	0.08	0.39
Method® HE Laundry Detergent, Free And Clear	1	9.88	8.82	0.00		4.10			
	2	0.46	0.43	0.00		0.00			
	3	-	-	-		3.96			
	Mean ± S.E.M	3.27 [a]	±	1.93	2.60	2.69 [a]	±	1.34	2.23
Seventh Generation® Natural 2X Concentrated Laundry Detergent	1	64.47	48.83	0.00		0.00			
	2	153.42	100.70	78.34		8.13			
	3	-	-	-		8.04			
	Mean ± S.E.M	74.29	±	21.01	59.06	5.39 [a]	±	2.70	4.48
Tide® Free & Gentle 2x Concentrated Laundry Detergent	1	24.16	20.91	0.00		0.03			
	2	49.23	45.52	31.98		1.34			
	3	-	-	-		0.00			
	Mean ± S.E.M	28.63 [a]	±	7.35	22.76	0.45 [a]	±	0.44	0.38
Tide® Powder Laundry Detergent with Bleach	1	0.00	0.00	0.00		0.08			
	2	0.00	0.00	0.00		0.00			
	3	-	-	-		0.64			
	Mean ± S.E.M	0.00 [a]	±	0.00	0.00	0.24 [a]	±	0.20	0.20

Laundry Additive	Experimental Replicate	1 ml of Laundry Additive to 1 ml of Blood			% Recovered	1 ml Diluted Laundry Additive to 1 ml of Blood			% Recovered
		Technical Replicate (ng/µl)				Technical Replicate(ng/µl)			
		1	2	3		1	2	3	
Tide® HE Laundry Detergent, Original Scent	1	38.25	24.61	6.31		0.10			
	2	25.33	15.01	2.57		0.00			
	3	-	-	-		0.01			
	Mean ± S.E.M	18.68 [a]	±	5.44	14.85	0.04 [a]	±	0.03	0.03
Tide® PODS™ Spring Meadow	1	70.20	21.35	2.35		0.07			
	2	14.04	7.88	7.55		31.28			
	3	-	-	-		0.00			
	Mean ± S.E.M	20.56 [a]	±	10.28	16.34	10.45 [a]	±	10.41	8.68
Wisk® 2X Liquid Detergent, Deep Clean	1	0	0	0		1.11			
	2	0.43	0	0		0.84			
	3	-	-	-		0.92			
	Mean ± S.E.M	0.07	±	0.07	0.06	0.96 [a]	±	0.08	0.80
Bounce® Fabric Softener Sheet, Outdoor Fresh	1	0.69	0.61	0.47		0.00			
	2	5.66	4.68	4.52		0.01			
	3	-	-	-		1.93			
	Mean ± S.E.M	2.77 [a]	±	0.99	2.20	0.65 [a]	±	0.64	0.54
Tide® To Go Pen	1	25.16	13.7	6.8		0.00			
	2	7.98	7.15	5.76		0.01			
	3	-	-	-		1.93			
	Mean ± S.E.M	11.09 [a]	±	3.04	8.82	0.65 [a]	±	0.64	0.54
OxiClean® Max Force® Laundry Stain Remover Spray [b]	1	17.96	17.65	7.17		2.18			
	2	23.54	7.56	4.43		2.17			
	3	-	-	-		1.73			
	Mean ± S.E.M	13.05 [a]	±	3.13	10.37	2.03 [a]	±	0.15	1.68

Laundry Additive	Experimental Replicate	1 ml of Laundry Additive to 1 ml of Blood			% Recovered	1 ml Diluted Laundry Additive to 1 ml of Blood			% Recovered
		Technical Replicate (ng/µl)				Technical Replicate(ng/µl)			
		1	2	3		1	2	3	
Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent	1	14.6	14.36	11.6		18.70			
	2	53.04	42.04	41.19		0.00			
	3	-	-	-		14.77			
	Mean ± S.E.M	29.47 [a]	±	7.35	23.43	11.16 [a]	±	5.69	9.26
Seventh Generation® Free & Clear Natural Laundry Detergent, Unscented	1	0	0	0		0.00			
	2	0	0	0		3.94			
	3	-	-	-					
	Mean ± S.E.M	0.00 [a]	±	0.00	0.00	1.97 [a]	±	1.97	1.64
Detergents Combined	Mean ± S.E.M	16.76	±	4.58	13.321	2.68	±	1.56	2.22

Table 5: Autosomal STR profiles from blood exposed to undiluted laundry additives

Loci	Laundry Additive Description						
	Ajax® 2x Ultra Liquid Laundry Detergent with Bleach Alternative, 50 oz.	Ultra Gain® 2x Liquid Laundry Detergent, Original , 50 oz Detergent	# 8 All Natural Detergent Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz	Tide® Free & Gentle 2x Concentrated Laundry Detergent, 100 fl. Oz	# 16 Active Oxygen Stain Remover Spray	Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, 50 oz.	Subject 1's Blood
D10S1248	13, 16	13, 16	13, 16	13, 16	13, 16		13, 16
vWA	17, 18	17, 18	17, 18	17, 18	17, 18		17, 18
D16S539	11, 12	11, 12	11, 12	11, 12	11, 12		11, 12
D2S1338	25, 26	25, 26	25, 26	25, 26	25, 26		25, 26
Amel	X, X	X, X	X, X	X, X	X, X	X,X	X, X
D8S1179	12, 13	12, 13	12, 13	12, 13	12, 13	13	12, 13
D21S11	29, 31	29, 31	29, 31	29, 31	29, 31	29, 31	29, 31
D18S51	13, 15	13, 15	13, 15	13, 15	13, 15		13, 15
D22S1045	16, 16	16, 16	16, 16	16, 16	16, 16	16	16, 16
D19S433	14, 15	14, 15	14, 15	14, 15	14, 15		14, 15
TH01	9, 9.3	9, 9.3	9, 9.3	9, 9.3	9, 9.3		9, 9.3
FGA	18, 25	18, 25	18, 25	18, 25	18, 25	18, 25	18, 25
D2S441	10, 14	10, 14	10, 14	10, 14	10, 14	10, 14*	10, 14
D3S1358	17, 18	17, 18	17, 18	17, 18	17, 18		17, 18
D1S1656	14, 18.3	14, 18.3	14, 18.3	14, 18.3	14, 18.3		14, 18.3
D12S391	24, 24	24, 24	24, 24	24, 24	24, 24		24, 24

Table 6: Autosomal STR profiles from blood exposed to diluted laundry additives

Loci	Laundry Additive Description		
	Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.	Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, 50 oz.	Subject 1's Blood
D10S1248	13, 16	13, 16	13, 16
vWA	17, 18	17, 18	17, 18
D16S539	11, 12	11, 12	11, 12
D2S1338	25, 26	25, 26	25, 26
Amel	X, X	X, X	X, X
D8S1179	12, 13	12, 13	12, 13
D21S11	29, 31	29, 31	29, 31
D18S51	13, 15	13, 15	13, 15
D22S1045	16, 16	16, 16	16, 16
D19S433	14, 15	14, 15	14, 15
TH01	9, 9.3	9, 9.3	9, 9.3
FGA	18, 25	18, 25	18, 25
D2S441	10, 14	10, 14	10, 14
D3S1358	17, 18	17, 18	17, 18
D1S1656	14, 18.3	14, 18.3	14, 18.3
D12S391	24, 24	24, 24	24, 24

For the full-scale test, the recovered DNA quantity values of blood combined with laundry additives were used to identify the most and least effective laundry additives on removing or denaturing DNA for a full-scale test. 'Downy® Non Concentrated Fabric Softener, April Fresh' was shown to be the most effective laundry additive and have the smallest range ($\overline{x}$ = 0.01 ± .001 ng/µl). 'Method® HE Laundry Detergent, Free and Clear' was also selected because it was the most effective detergent with the smallest range ($\overline{x}$ = 3.27±1.93 ng/µl). In addition, 'Seventh Generation® Natural 2X Concentrated Laundry Detergent' was selected due to it being the least effective detergent with the smallest range ($\overline{x}$ = 74.29 ± 21.01 ng/µl) (Table 4). Quantifiable DNA was found from all three-laundry types, with Downy® having the smallest concentrations and Seventh Generation having the highest concentrations (Table 7).

Table 7: Quantity of DNA (ng/µl) recovered after laundering using the most, lease defective detergent, and most effective laundry additive.

Laundry Additives		Experimental Replicate 1	2	3	% Recovered
Laundered Shirt Without Additives.	Mean ± S.E.M	0.64 14.81	41.24 ±	2.54 13.23	-
Downy® Non Concentrated Fabric Softener, April Fresh, 64 oz.	Mean ± S.E.M	0.07 0.07	0.06 ±	0.07 0.00	0.45
Method® HE Laundry Detergent, Free And Clear, 20 oz.	Mean ± S.E.M	0.43 0.41	0.45 ±	0.34 0.03	2.75
Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.	Mean ± S.E.M	0.3 0.43	0.61 ±	0.38 0.09	2.90

DISCUSSION

It is generally assumed that applying detergent without diluting it on a bloodstain would be the most effective method for removing blood. What is surprising though is that the diluted laundry additives on average had 11% less recovery of DNA than the undiluted additives. The raw data would support the idea that diluted additives would be more effective, and several pairs of detergents had diluted and undiluted means that looked very dissimilar; however only three of the additives were significantly different between concentrations.

Both ‘seventh generation detergent’ and ‘all HE 2x Oxi-active’, at undiluted strength, did not show strong evidence to suggest that the recovery rates are different than combining blood with water. Whereas all of the diluted laundry additives showed strong evidence to suggest that they are more effective in reducing the amount of DNA. This suggests that diluting laundry additives to the recommended amount by the manufacturer would be more effective at hindering the recovery of DNA than water.

Using q-values two out of the 18 laundry additives analysis failed to reject the null hypothesis that exposing blood to undiluted laundry additives will have no effect on the recovery rate of DNA (see Table 26 in Appendix A). Using the q-value all of the diluted laundry additive results suggest strong evidence to reject the null hypothesis that there is no effect on the recovery rate of DNA between using no laundry additives and using one of the 18 diluted laundry additives (see Table 27 in Appendix A).

The averages of recovered DNA for the diluted laundry additive were smaller than the average recovery rates for the undiluted laundry additives in 13 of the 18 laundry additives tested, which supports that idea that just using detergent will hinder the recoverability of DNA. However, diluting the laundry additive to the proper concentration works more efficiently in

most cases. Only three of the eighteen laundry additives tested showed a significant difference in recovery rates when comparing the two concentrations. Using the p-values from the ANOVA with a Bonferroni correction and a $\alpha = 0.013$ level of significance there is not sufficient evidence to reject the null hypothesis that exposing blood to diluted or undiluted laundry additives will have no effect on the recovery rate of DNA (see Table 28 in Appendix A).

After reviewing the data and the techniques used, there were some modifications that would be suggested for further study on the effects of laundry additives on DNA. For example, due to the viscosity of certain additives at full strength, some of the solutions were not consistent throughout even after agitation. This possibly results in different concentrations of blood to be transferred, subsequently causing a wider standard deviation and range in the results than expected. A solution to this problem would be to use larger volumes for starting material for extraction to counteract the possibility of altering the concentration of DNA material.

The laundry additives may have been more effective when diluted in water due to the steric hindrance of the chemicals reacting with the components of the cells. Further studies could be done with different additives, incubation temperatures, or allowing more time for the solution to react. Furthermore, some of the organic ingredients in laundry additives may have dissolved in the organic layer of the extraction procedure and consequently could have affected the quantification of DNA. This would explain the over amplification of samples during STR amplification. A different extraction technique could address the dissolving of organic ingredients in the organic layer.

Previous studies have focused on sodium percarbonate as a laundry additive ingredient hindering the identification of bloodstains (Castello, Frances, & Corella, 2009). However, the results from this study suggest that forensic science professionals should be as concerned if not

more concerned about the use of concentrated detergents and softeners since they were the more effective at removing the blood then additives with sodium percarbonate (Table 4). This study extends the work done by Castello et al. (2009), because it focuses on a wider range of available laundry additives (Table 2).

The third part of the laundry additive study simulated a garment being washed in personal use laundry machines that could be available to criminals, with the most and least effective detergents. The data showed that a suitable amount of DNA could be recovered from laundered clothing for profiling (Table 7) even with the most effective detergent. This experiment of laundering clothing with additives is more representative of crime scene evidence (State v. Pizzoferranto, 2005) than previous studies done by soaking stains in diluted detergent for two or more hours (Castello, Frances, & Verdu, 2012; 2010; Castello, Frances, & Corella, 2009).

Unfortunately, due to limited resources, DNA profiling was not conducted on the T-shirts from the full-scale test. However, the quantity recovered and Genotyping results from parts one and two suggest that the DNA profile would match the known profile of Subject 1. Further testing could be done to confirm that an accurate profile could be established

It was also found that laundry additives are the most effective at removing blood when used at the recommended amounts listed on the container. This is particularly useful, as investigators might not have considered sending a bloody garment for DNA testing that was saturated with laundry additives without this study.

CONCLUSIONS

This research shows that 16 laundry additives at undiluted strengths and 18 laundry additives at the manufacturers recommended concentration does significantly reduce the recovery of DNA. Even with the reduced volumes, though, it is not sufficient to prevent DNA profiling using traditional forensic techniques. Unexpectedly, the diluted additives were more effective than undiluted additives in 13 of the 18 laundry additives tested. However, only three of the laundry additives arithmetic means were significantly different between the two concentrations.

It is possible to use standardized techniques to isolate the DNA profile, but further work could be done to address the large variability in the quantifier results, which would subsequently improve the electropherogram results. Never the less, the data strongly suggests that DNA affected by laundry additives should still be tested for evidentiary value. This research is helpful for the forensic community to help readdress the stereotypes that surround DNA laden material that has been affected by laundering additives.

CHAPTER 3: THE EFFECTS OF DELAY BEFORE WASHING AND THE AMOUNT OF BLOOD DEPOSITED ON RECOVERY OF DNA FROM LAUNDERED CLOTHING

ABSTRACT

Perpetrators have used laundry machines to obscure evidence from crime scenes; sometimes they are not prompt, instigating a delay before laundering. This study focused on the amount of DNA recovered from garments washed immediately and at 1, 6, 12, and 24 hour intervals after a deposit of human blood. The data indicated that even with immediate washing, sufficient DNA was recoverable for profiling using a phenol-chloroform extraction. Additional research using two volumes of blood showed a visual increase in the amount of DNA recovered, but the results were not statistically significant. Even with small recovery values, the collected DNA profiles were consistent with the expected profiles of the donor DNA for this study. These results suggest that machine laundered DNA should be considered for sources of evidentiary value.

INTRODUCTION

In 2010 there were 157.0 million (US Census, 2015) females living in the United States. At some point, these women would have undoubtedly had to clean intentionally and unintentionally deposited blood during menstruation. Common lore and instructions that come with washing machines (Kenmore, 1999) and detergents describe a wide range of methods to remove blood from cloth. Most of the approaches have one thing in common, and that is to wash the garment as soon as possible. With all of this knowledge about specifically removing blood from cloth, and roughly 82% of households in the United States having access to laundering facilities (Cluett, Amann, Chou, & Osann, 2013), criminals may attempt to obscure blood-soiled clothing through machine laundering.

Even though laundered clothing is a possible source of evidence, the forensic community has limited studies on machine laundering effectiveness of hindering the recovery of DNA from bloodstained clothing. Unfortunately, DNA degrades over time, prompting several studies that focus on the recovery of DNA from bloodstains of different volumes and ages (Anderson, Hobbs, & Bishop, 2011; Bremmer, De Bruin, Van Gemert, Van Leeuwen, & Aalders, 2012; Foran, 2006; Anderson, Howard, Hobbs, & Clifton, 2005). However, with many studies focusing on the color of bloodstains, smear

resistance, or the percentage of RNA, researchers have not looked at the effects of ageing on DNA that has subsequently been washed.

Currently, there is no universal procedure for crime laboratory personnel to examine machine-laundered clothing even though the methods to remove blood are promoted by manufacturers of laundry additives and washing machines. Studies exist examining the persistence of blood, but none have attempted to see if a bloodstain would withstand the pressure and movement in the washer.

Pressure resistance research on human blood drying properties was done using droplets representing low velocity impact spatter (such as a gravitational droplets from a knife) on non-porous surfaces to see how they would be resistant to smearing (Ramsthaller et al., 2012). The results suggested that it took about 45 minutes of drying time before the blood droplets were unaffected by the pressure and movement (Ramsthaller et al., 2012). It was noticed that the blood droplets would dry from the outside towards the center and would leave a "skeletonized ring" of blood cells (Ramsthaller et al., 2012) if the fluid was not fully dry before it was disturbed. Ramsthaller and authors determined that the ring could be used as a method to estimate the time before the droplet was agitated.

Ramsthaller's work would suggest that the longer DNA laden material is in direct contact with the substrate, the more likely it is to stain, and consequently the harder it is

to completely remove. The skeletonized ring of a bloodstain may be resilient enough to withstand the laundering process if it is allowed to dry on the substrate before laundering. It could be argued that higher concentrations of DNA laden cells would be found in the rings rather than the rest of the agitated blood smear. Assuming that enough of the cells remained on the clothing, a DNA profile could be recovered from the laundered garment.

Liquids are easier to transfer than solids due to their physical properties. The length of time it takes for a bloodstain to dry could depend on the ambient temperature (Ramsthaller, Schmidt, Bux, Kalser, & Kettner, 2012), or the surface to volume area ratio of the bloodstain. These factors might affect the amount of blood transferred.

Different volumes and speeds of application will lead to unique dispersals of blood onto a substrate. At higher velocities, the blood droplets become smaller creating a larger surface area to volume ratio (see Figure 9). This ratio in turn would affect the soil's activation energy, which is defined as the energy needed for soils to leave a substrate and go into solution (Bajpai & Tyagi, 2007).

A **B** **C**

Figure 9: Examples of blood impact spatter at A) low velocity B) medium velocity C) high velocity.

In Low Velocity Impact (LVI) spatter (see Figure 9A), blood droplets may have a low activation energy. Since LVI spatter has a large volume per droplet, it could take longer to dry. Due to the increase in drying time, the undried blood could easily wash away. However, due to the volume of the blood spatter, the stain may take some time to be fully obliterated. On the other end of the spectrum, in High Velocity Impact (HVI) spatter (see Figure 8C), the blood droplets may dry faster, and as a result have a high activation energy. Although once the activation energy requirements are met, the stains are more likely to be easily obliterated because the droplets do not have a lot of volume.

The distribution of blood on a surface can be affected by the exterior of the substrate. Non-porous surfaces will cause blood droplets to sit on top of the surface and take longer to dry (MacDonell, 2005). Porous surfaces, such as clothing, allow the blood

to seep into material, which increases the blood droplet's surface area (MacDonell, 2005). The increase in surface area would allow the blood droplet to dry faster.

Modern clothing are made out of natural and synthetic materials. Wool, linen, and other natural fibers have cuticles that overlap which may retain foreign cellular material (see Figure 10). In contrast, synthetic materials, due to the manufacturing process, have fibers with a smooth outside (Tascan & Edward, 2008), which may not be able to hold cells as well as natural fibers.

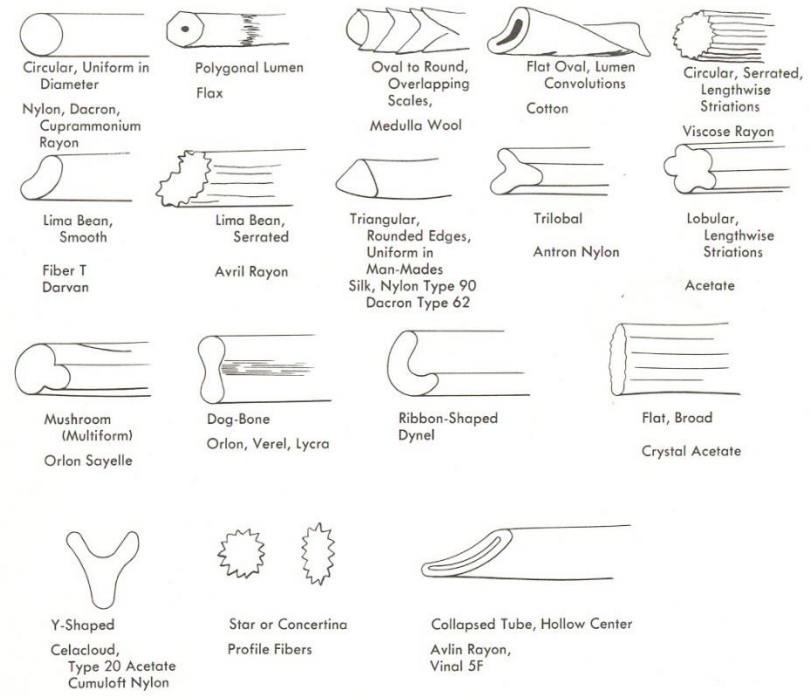

Figure 10: Cross-section of synthetic and natural fibers (Hollen, Seddler, & Langford, 1979)

For example, research using all cotton clothing showed that human spermatozoa could withstand the machine laundering process (Kafarowski, Lyon, & Sloan, 1996). To show the spermatozoa could be retained, sperm cells were deposited on cotton panties after normal intercourse and laundered. Small cuttings of the laundered panties were collected and the sperm cells were removed, stained and counted (Kafarowski, Lyon, & Sloan, 1996). Unfortunately, DNA testing was not conducted to see if a profile could be generated. On average, 1-2 sperm cells were found on each cutting after washing (Kafarowski, Lyon, & Sloan, 1996). Kafarowski's et al. (1996) work supports the theory that sperm cells could withstand the rigors of laundering and suggests that other types of cells could be retained on articles in the wash in a similar manner.

The medical legal community has treated laundered evidence with caution due to factors such as: how a bloodstain dries, bloodstain shape and volume, fiber type, and limited studies on the transfer of DNA laden material. Doubt over the quality of the DNA has caused it to be ignored as a possible source of evidence in criminal investigations. The existence of laundered evidence and lack of information to process it, suggests a need to study the effects of laundering dried DNA evidence.

With the possibility of gathering a DNA profile from laundered clothing, two research questions were tested in this study: 1) What are the effects of a delay between

deposit and laundering on amount of recoverable DNA for a DNA profile? 2) Would the amount of blood deposited affect the amount of DNA recovered?

The amount of time between deposit and laundering might result in an increase of dried blood. The dried blood could take a higher activation energy to remove (Bajpai & Tyagi, 2007) than blood in a liquid state, and could result in higher amounts of recovered DNA material. Testing this concept may establish standards that could be used to determine a possible progression of events during a crime. This could be done by analyzing the presence of a 'skeletonized ring' (Ramsthaller *et al.* 2012) or the amount of DNA recovered.

Standardized tests that vary the amounts of blood as source material could give guidance to investigators to estimate the amount of blood originally deposited on a laundered article of clothing. This in turn could help collaborate or discredit a suspect's testimony of what may have occurred.

To address these concerns the following research hypotheses were developed:

1. There is no difference between using 5ml or 10 ml of blood on the recovery rate of DNA.
2. For any one given time delay, there is no significant difference between each delay before laundering on the recovery rate of DNA.
3. Laundering will obstruct the production of a DNA profile.

METHODS

Two experiments were used to test the effect of a delay before washing and different amounts of blood on the recovery of DNA from laundered clothing. The delay times were selected to show the effects of time over a 24-hour period. To see the necessary amount of blood needed to recover DNA from laundered clothing in the wash two volumes were selected to represent possible blood deposit events.

A publicly available washing machine was used to launder garments to simulate possible laundering events. The machines used were a 2003 top loading Kenmore (model number 20712990) washer and Kenmore (model number 110.60902990) dryer. The location of the machines has one exterior door and keypad locked interior entrances and is accessible to professionals at Wright State University (see Figure 15 in Appendix A) and as such would be classed as an on premise laundering facility (Bajpai & Tyagi, 2007). The machines are typically used to clean lab coats or other cloth materials used on campus. Before use, the machines were sanitized by spraying all available surfaces with a 20 % bleach solution and allowed to stay moist for 20 minutes.

Sample Preparation

A medium sized 100 % cotton T-shirt (Gildan 5000) was used as the substrate for the deposit of blood. A piece of autoclave tape was placed within the folds of each item inside an autoclave bag and a piece of autoclave tape was placed on the outside of the

bag. The bags were autoclaved with no more than one layer of clothing on the shelf at 120 ^{o}C for 20 minutes and then allowed to come to room temperature. The fabric was stored in the autoclave bag until use and was opened within a class two biological safety cabinet. If the autoclave tape that was placed within the clothing did not show a positive reaction for sterilization, then the clothing was not used. A control study using this method of preparation for sterilization was completed (see Table 29 in Appendix A).

Human blood used for this study was obtained from the Community Blood Center/Community Tissue Services (CBC/CTS) in Dayton, Ohio. The blood was donated by volunteers in the Dayton, Ohio, USA area. All donors were given written notice, and consented to the use of their blood in unspecified research at the time of donation. No demographics were released to the researcher at any time, and the researcher did not have any contact with the donors. A genotype profile was generated for comparison of samples collected (Table 8).

The prepared shirts were spread out on a clean sheet of bench protector paper with the plastic side up in a clear plastic container (see Figure 7 in Chapter 2) inside a Biological Safety Cabinet (BSC). The blood was transferred from the donor bag to a glass container and stirred. The blood was applied using a manual pipette and disposable tips. The distance between the pipette tip and the clothing was 10 cm.

Table 8: Genotype profile for Subjects 2 and 3

Loci	Subject 2	Subject 3
D10S1248	13, 13	13, 14
vWA	17, 19	16, 18
D16S539	9, 12	11, 14
D2S1338	23, 23	17, 18
Amel	X, X	X, Y
D8S1179	12, 16	13, 14
D21S11	31.2, 31.2	27, 31.2
D18S51	19, 20	13, 15
D22S1045	11, 16	16, 16
D19S433	14, 16	13, 13
TH01	7, 9.3	8, 9
FGA	23, 24	19, 22
D2S441	14, 14	10, 14
D3S1358	15, 17	14, 15
D1S1656	11, 12	17.3, 18.3
D12S391	21, 22	17, 20

Between each load of laundry, a bleach load was used to clean out the washer and reduce possible contamination between loads. A 20% bleach solution was sprayed on the inside of the washer and dryer and allowed to remain moist for 20 minutes to eliminate DNA from the inside surfaces. Then a T-shirt was added to the washer with 709 ml of bleach (maximum recommended amount by the manufacturer) and run on “medium/large load” water level, “hot/cold” water temperature “heavy duty” speed for 10 minutes. Once the wash cycles were complete, the shirt was transferred to the dryer and run on “cotton/high” for 60 minutes.

Part One: Delay Between Deposit of Blood and Laundering

To test how the time delay would affect the DNA, Subject 3's blood (5 ml) was applied to nine shirts. Shirts 1, 2, and 3 were washed immediately. Shirts 4, 5, and 6 had a one-hour delay between deposit and washing. Shirt 7 had a six-hour delay, Shirt 8 had a twelve-hour delay, and Shirt 9 had a twenty-four hour delay. All time delays were conducted by placing the shirts on the counters in the lab on top of their bench protector paper used to deposit the blood at 22 ^{o}C in the lab without any protection. The shirts were washed at "medium" water level with a "warm, warm" water temperature on the "regular" speed setting for 10 minutes. The shirt was transferred to the dryer set to "normal/perm press" temperature for 60 minutes. Between testing loads, a bleach load was used to sanitize the washer and dryer. Fabric cuttings (1 cm^2) were cut from the center of the shirts and stored in separate 1.5 ml test tubes for extraction. The rest of the shirt was stored in labeled paper evidence bags.

Part Two: Amount of Blood Deposited

To test if the amount of blood deposited on a soiled shirt affected the amount of DNA that was recoverable, either 5 ml or 10 ml of blood of was deposited on a shirt. There were three shirts washed for each volume of blood. The shirts were immediately washed with a standard load on "medium" water level with a "warm, warm" water temperature on the "regular" speed setting for 10 minutes The shirt was transferred to the

dryer that was set to 'normal/perm press' temperature for 60 minutes. A bleach load was used to clean the washer between each load. Fabric cuttings (1 cm^2) were cut from the center of each T-shirt and stored in separate 1.5 ml test tubes for extraction (N=6). Each load was stored in labeled paper evidence bags.

Extraction

This procedure was adapted from AmpFlSTR Profiler Plus PCR Amplification Kit User's Manual (Applied Biosystems, 2012) and Forensic DNA Analysis: A Laboratory Manual (McClintock, 2008). All samples collected for extraction were combined with 500µl of digest buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, pH 7.5) and 15 µl of 10 mg/ ml Proteinase K (Applied Biosystems, 2012). The solution was vortexed for 15 seconds and incubated between eight hours at 56 ^{o}C. Phenol-Chloroform Isoamyl Alcohol (PCIA) (500µl) was added to each tube and vortexed for 15 seconds and centrifuged at 1500 rpm for five minutes. The aqueous layer then was transferred to a new test tube and the PICA step was repeated two more times. Ethanol (1 ml) was added to each tube and incubated for 30 minutes at 0 ^{o}C. The samples were centrifuged for 15 min at 1500 rpm and the liquids removed. Ethanol (1 ml) was added to each tube and vortexed for 15 seconds. The samples were centrifuged for 15 min at 1500 rpm and the liquids removed. The test tubes were allowed to air dry inside of a

biological safety cabinet. 36µl of 1x TE-Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) was added to each sample and stored at 0 ºC (McClintock, 2008).

Quantification

Extracted DNA was quantified using the standard protocol from Applied Biosystems for the Human Quantifier Kit User Manual (Applied Biosystems, 2012). ABI Prism® 7900HT Sequence Detection System (Applied Biosystems, 2001) was used to collect data. Either sample (2 µl) or standard (2 µl) was added to separate wells in a MicroAmp Optical 96-Well Reaction Plate (Life Technologies). Master Mix (23 µl made of 10.5 µl Quantifier Human Primer mix, 12.5 µl Quantifier PCR Reaction mix) was added to each well and the plate was sealed with optical adhesive cover. Thermal cycling parameters consisted of stage one at 95ºC for 10min, stage two was 95 ºC for 15 seconds then 60 ºC for 1 minute with 40 repeats. The IPC and Quantifier Human detectors were set with a standard curve and the 9600 emulation option on.

DNA standard series was made using a stepwise dilution of Quantifiler™ Human DNA Standard with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0 (McClintock, 2008)) following the manufacturer's recommendations. A series of eight standards were run in duplicate with the concentrations ranging from 0.023 ng/µl to 50 ng/µl.

STR Amplification

Using the quantification data, 10 ng of the samples was transferred and air-dried in PCR tubes for shipment to the University College of London in London, England. Polymerase Chain reaction (PCR) was conducted on DNA extracts using AmpFlSTR® NGM™ PCR Amplification Kit (Applied Biosystems, 2012). Samples were suspended to a concentration of 0.5-1 ng/µl, with 1µl of each sample added to separate PCR test tubes with 15 µl of Master Mix (10.0 µl AmpFlSTR NGM Master Mix, 5.0 µl AmpFlSTR NGM Primer Set). Thermal cycling parameters consisted of 1 minute at 95 ^{o}C, followed by 29-30 cycles of 20 seconds of denaturation at 95 ^{o}C and 3 minutes of annealing at 59 ^{o}C with a final extension for 10 minutes at 60 ^{o}C.

STR Capillary Electrophoresis

Five or ten µl of standard or amplified DNA was added to separate wells on a 384 well plate and centrifuged of 5 seconds at 1500 rpm. Samples were analyzed on the Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, 2010). The run parameters consisted for 5 second injection period, 3 kV per 5 seconds injection voltage, 15.0 kV run voltage, for 120 min, as a 'standard run', with a capillary length of 50cm.

Data Analysis

Quantification Data from the 7900HT Sequence Detection System was analyzed using Statistical Package for Social Sciences®, version 21.0 (IBM, 2012). To test for

significant differences between values and control for a type one error an ANOVA Test with a post-hoc Tukey multiple comparison test was used. This can be calculated using the equation:

$$q = \frac{Control\ Mean - \ The\ mean\ of\ the\ test\ variable\ set}{\sqrt{Mean\ squared\ \left(\frac{1}{number\ per\ treatment}\right)}}$$

In order to meet the required assumptions of normality and constant variance DNA recovered data points were log transformed. The percentage values were calculated by dividing the arithmetic mean of a particular sample by the arithmetic mean of the positive control.

Capillary electrophoresis data was analyzed using GeneMapper, version 4.0 (Applied Biosystems, 2006). Recorded alleles exceeded a minimum threshold of 50 relative florescence units (RFUs). If a minor profile was observed then the minor profile was identified by using the assumption that certain alleles were indicative of the original planned profile and had normal morphology for alleles rather than artifacts.

RESULTS

The average recovery of DNA from the shirts increased from 0.15 ng/µl ± 0.02 to 9.63 ng/µl ± 3.83 when the time between deposit and washing was increased from zero hours to 24 hours (see Table 9 and Figure 11). This is an increase from 0.12 % to 8.0% of the total amount of blood projected to be in the original sample (see Table 9). All of the tested time-periods resulted in a lower recovery rate then the un-washed positive control.

Table 9: Recovered values of DNA from shirt with 5 ml of blood applied and allowed to air dry for 0, 1, 6, 12 and 24 hours before laundering.

Time	Experimental Replicate	Technical Replicate (ng / µl)						% Recovered
		1	2	3	Mean	±	S.E.M	
Positive	1	114.37	138.10	108.90	120.45	±	8.96	-
Immediate washing	1	0.21	0.09	0.10	0.13	±	0.04	
	2	0.16	0.13	0.08	0.12	±	0.02	
	3	0.21	0.19	0.18	0.19	±	0.01	
	Mean ± S.E.M				0.15	±	0.02	0.12
One hour delay	1	4.77	2.02	0.92	2.57	±	1.15	
	2	0.84	0.41	0.52	0.59	±	0.13	
	3	1.08	0.71	0.88	0.89	±	0.11	
	Mean ± S.E.M				1.35	±	0.62	1.12
Six hour delay	1	1.12	2.27	0.90	1.43	±	0.43	1.19
Twelve Hour delay	1	6.12	6.25	3.56	5.31	±	0.88	4.41
Twenty-Four Hour delay	1	17.28	6.25	5.37	9.63	±	3.83	8.00

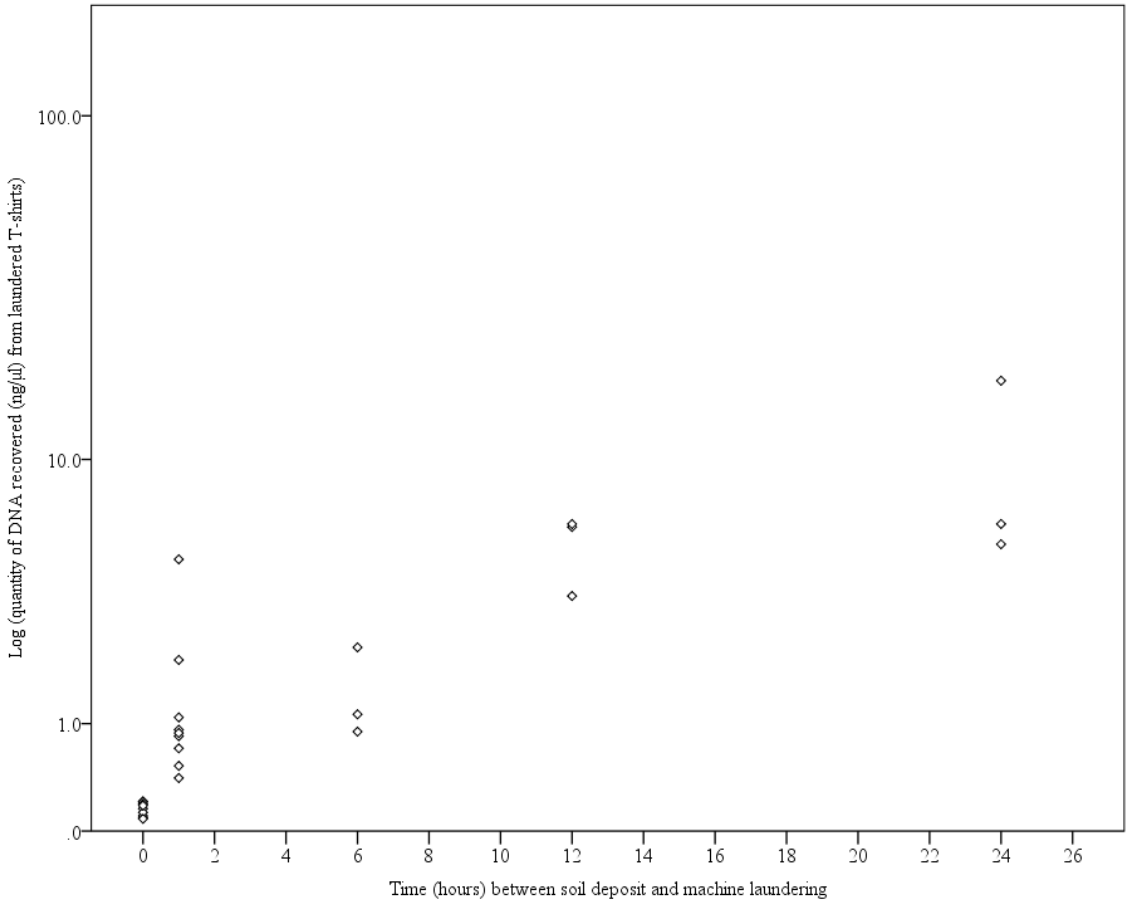

Figure 11: A comparison of the quantity of DNA recovered (ng/µl) from laundered T-shirts soiled with 5ml of blood and allowed to dry for 0,1, 6,12, and 24 hours before machine laundering.

Except between zero and one hour delay before laundering, there were no significant differences between two consecutive time intervals ($p<0.05$) (Table 10).

Table 10: Tukey multiple comparison of DNA recovery values from shirts with different time intervals between soiling and laundering. [a] comparisons are significant at the 0.05 level

Difference Of Means ± 95% Confidence Limit Between Two Delay Intervals

Delay Intervals (Hours)	Delay Intervals (Hours)				
	0	1	6	12	24
0	-	-1.9693[a] ±1.8375	-2.2375 ±2.5986	-3.5996[a] ±2.5986	-4.0826[a] ±2.5986
1	1.9693[a] ±1.8375	-	-0.2683 ±2.5987	-1.6303 ±2.5986	-2.1133 ±2.5986
6	2.2375 ±2.5987	0.2683 ±2.5986	-	-1.3621 ±3.1827	-1.8451 ±3.1827
12	3.5996[a] ±2.5986	1.6303 ±2.5987	1.3621 ±3.1826	-	-0.483 ±3.1827
24	4.0826[a] ±2.5987	2.1133 ±2.587	1.8451 ±3.1826	0.483 ±3.1837	-

DNA profiling on the delay between deposit and washing samples were consistent with Subject 2. Split peaks were observed in the one-hour delay in the FGA, D2S441, D3S1358, D1S1656 and D12S391 loci. A degraded minor profile found on an immediate wash (0 hour) T-shirt was consistent with a known DNA profile from lab personnel (see Table 11, for electropherograms see Figure 21 to Figure 23 in Appendix B).

Table 11: Autosomal STR profiles from laundered T-shirts with a 0, 1 and 24 hour delay before washing.*some artifacts and unusual peak shape observed for allele position.

Loci	Amount of Time Before Laundering				Subject 2	Lab Personnel Control
	0 hour		1 hour	24 hours		
	Major	Minor	Single	Single		
D10S1248	13, 13	15, 16	13, 13	13, 13	13, 13	15, 16
vWA	17, 19	18	17, 19	17, 19	17, 19	16, 18
D16S539	9, 12	13*	9, 12	9, 12	9, 12	12, 13
D2S1338	23, 23	20	23, 23	23, 23	23, 23	20, 25
Amel	X, X	X, Y	X, X	X, X	X, X	X, Y
D8S1179	12, 16	13, 15	12, 16	12, 16	12, 16	13, 15
D21S11	31.2, 31.2	27, 30	31.2, 31.2	31.2, 31.2	31.2, 31.2	27, 30
D18S51	19, 20	16	19, 20	19, 20	19, 20	16, 19
D22S1045	11, 16	17*	11, 16	11, 16	11, 16	15, 16
D19S433	14, 16		14, 16	14, 16	14, 16	13, 14
TH01	7, 9.3	8, 9	7, 9.3	7, 9.3	7, 9.3	8, 9
FGA	23, 24	21	23, 24	23, 24	23, 24	21, 23
D2S441	14, 14	10,10	14, 14	14, 14	14, 14	10, 10
D3S1358	15, 17	16,16	15, 17	15, 17	15, 17	16, 16
D1S1656	11, 12	14, 15	11, 12	11, 12	11, 12	14, 15
D12S391	21, 22		21, 22	21, 22	21, 22	21, 22

The average amount of DNA recovered decreased from 17.49 ng/µl ± 10.09 to 14.81 ng/µl ± 13.23 when the volume of blood deposited was increased from 5 ml to 10 ml before laundering (see Table 12). The shirt with 5ml of blood deposited had a recovery rate of 14.5 % whereas the 10 ml of blood had a recovery rate of 12.2 %. Although the difference was not significant (p= 0.252) when comparing the mean of

recovered DNA from either 5ml or 10ml of starting material (see Table 13 and Figure 12).

Table 12: Recovery values of DNA from shirt with either 5ml or 10ml of human blood

Item	Experimental Replicate	Unwashed (ng/µl)	5ml (pg/µl)	10ml (pg/µl)
Blood Stained Shirt	1	114.37	35.11	2.54
	2	138.10	17.21	41.24
	3	108.90	0.17	0.64
	Mean ± S.E.M	120.45 ± 8.96	17.49 ± 10.09	14.81 ± 13.23
Percentage recovered		-	14.5	12.2

Table 13: Comparison of the means of total amount of DNA recovered between loads with either 5ml and 10ml of starting material. [a] Computed using Alpha = 0.05

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial ETA Squared	Noncent. Parameter	Observed Power [a]
Corrected model	0.081	13	0.006	2.49	0.02	0.545	32.3	0.898
Intercept	0.014	1	0.014	5.70	0.02	0.174	5.696	0.634
Amount	0.003	1	0.003	1.38	0.25	0.048	1.373	0.204
Error	0.068	27	0.003					
Total	0.163	41						
Corrected Total	0.148	40						

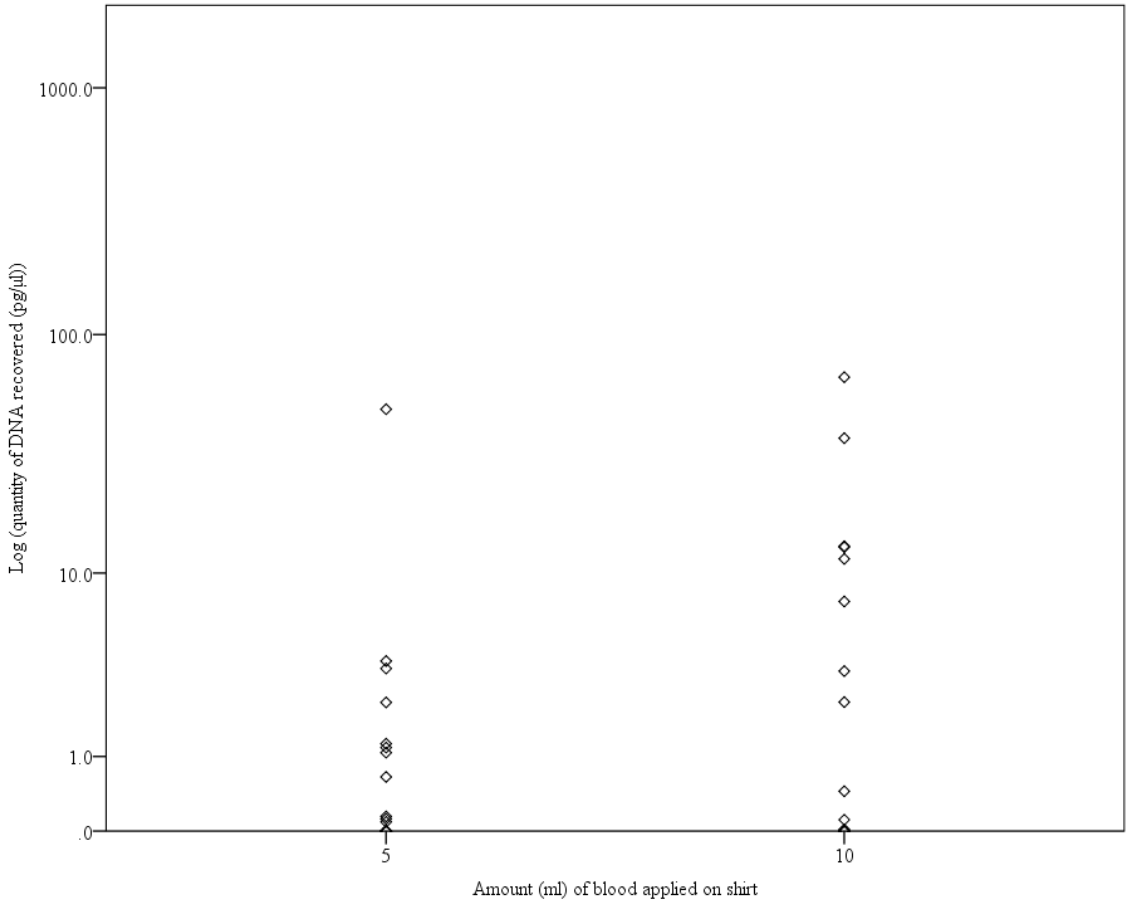

Figure 12: A comparison of the quantity of DNA recovered (pg/µl) from articles with transfer during machine laundering with a T-shirt soiled with either 5 or 10 ml of blood.

Table 14: Autosomal STR profiles from laundered T-shirts after laundering with either 5ml or 10ml of blood.

Loci	Subject 3	5 ml of Blood Deposited Before Washing	10 ml of Blood Deposited Before Washing
D10S1248	13, 14	13, 14	13, 14
vWA	16, 18	16, 18	16, 18
D16S539	11, 14	11, 14	11, 14
D2S1338	17, 18	17, 18	17, 18
Amel	X, Y	X, Y	X, Y
D8S1179	13, 14	13, 14	13, 14
D21S11	27, 31.2	27, 31.2	27, 31.2
D18S51	13, 15	13, 15	13, 15
D22S1045	16, 16	16, 16	16, 16
D19S433	13, 13	13, 13	13, 13
2TH01	8, 9	8, 9	8, 9
FGA	19, 22	19, 22	19, 22
D2S441	10, 14	10, 14	10, 14
D3S1358	14, 15	14, 15	14, 15
D1S1656	17.3, 18.3	17.3, 18.3	17.3, 18.3
D12S391	17, 20	17, 20	17, 20

DISCUSSION

There was a decrease in the amount of DNA recovered from the bloodstain when laundered. This result is not particularly surprising since the public specifically uses machine laundering to remove stains. However, even after washing, the extracted DNA was of sufficient quantity for profiling. When the DNA profile was produced, the findings were consistent with the expected profile of Subject 3.

The results support that the longer the blood is in contact with the shirt, the more DNA is recovered. This information agrees with layperson knowledge and instructions that come with laundry machines in that garments should be washed as soon as possible if the stain is to be removed (Kenmore, 1999). When examining the shirt with a twelve-hour delay a faint thin outline (skeleton ring) of the original bloodstain was visible after laundering, which is consistent with Ramsther's (2012) work on how blood droplets dry. Nevertheless, even with immediate laundering of the bloodstain, there was enough DNA to profile. The DNA profiles were consistent with Subject 3. This information provides data to reject the hypothesis that laundering will obstruct the production of a DNA profile.

Due to limited increments for the time delay between deposit and washing, and having a weak positive correlation without statistically distinguishable time intervals, a definitive timeline for a crime could not be established. Although a very rough estimate

of time could be theoretically given, factors like the amount of blood, environment, and substrate could affect the retention rate. Further research should use smaller increments of time between deposit and laundering and extend the number of time intervals to see if the recovery values plateau. Although samples were taken from the same areas within clothing, the redistribution of blood cells due to the machine laundering might have caused larger than expected standard deviation and range seen in the quantitative PCR data. Further research could use larger sample cuttings or combine multiple samples from the same item. Some over amplification was observed on the electropherograms of the one-hour delay samples, this is probability due to a miscalculation of the results from the quantitative PCR results.

An unintentional minor profile found in the immediate washing was found to be consistent with a profile from another researcher's experiment in the lab. However, it does show that a mixed profile can also be recovered from a mixture of two or more DNA sources after it has been laundered. Further experimentation of this knowledge could be used to establish who was wearing the shirt and the person who deposited blood.

The result for first part of this study rejects the hypothesis that the delay between deposit and washing does not affect the amount of DNA recovered. These data can help the forensic science community establish rough estimates for timelines and understand the context of laundered DNA better. These data also establishes credence to

the public opinion of washing soiled clothing immediately to reduce the likelihood of a stain.

The results show that by using larger starting amounts of blood, the amount of recoverable DNA does increase. This fits with the general understanding that by increasing the starting material the more likely a DNA profile can be obtained. Establishing the original amount deposited would be useful in collaborating a person of interest's testimony. Unfortunately, the data currently does not statistically support the possibility of a linear regression to numerically evaluate the amount of blood deposited before laundering. Therefore, this data supports the hypothesis that there is no difference between using 5ml or 10 ml of blood on the recovery rate of DNA.

This knowledge may lead to testing laundered clothing for DNA profiling more frequently due to the possibility of obtaining a suspect profile. This information could also help investigators understand the evidentiary value in collecting and maintaining laundered bloody clothing, and subsequent analysis.

CONCLUSIONS

This research shows that laundering bloody clothing does significantly reduce the recovery of DNA. Even with the reduced volumes, it is not sufficient to hinder DNA profiling using traditional forensic techniques. Additionally more DNA is recovered if the blood is allowed to dry on the clothing before laundering. Even though there appeared to be a positive correlation between the amount of DNA recovered and the original volume, the difference was not significant, which prevents using the amount recovered to determine the original volume deposited. Although, this limitation may be due to limited volumes examined.

It is possible to use standardized techniques to isolate the DNA profile, but further work could be done to address the large variability in the quantifier results, which would subsequently improve the electropherogram results. Additionally sample size may be a factor and may be addressed using larger cuttings or multiple cuttings from the same article.

CHAPTER 4: TRANSFER OF DNA WITHIN A PRIMARY LOAD AND TO A SECONDARY OR TERTIARY LOAD.

ABSTRACT

This research addressed the validity of using blood-soiled laundered clothing as possible source of evidence in a criminal case. This was studied using the recoverability of DNA from laundered clothing within a primary load, containing a blood stained shirt, and subsequent loads. DNA was recovered in all loads; however, there is a significant decrease in amount of DNA collected from a primary load compared to a secondary load (9.2 %), and from a secondary to a tertiary load (0.31 %). Additional testing on the garments within a primary load showed that DNA material could be transferred from a bloodstained garment to another garment. Furthermore, if the originally stained clothing were indistinguishable from all of the garments in the same load, a towel or a sock would, on average, have the highest amounts of DNA material of the initially unstained apparel.

INTRODUCTION

When a criminal investigator discovers blood in a washing machine, it would be important to know the potential of it being innocently transferred blood, before alleging that a crime had occurred. If the original source of DNA is not apparent or available, it would be prudent to know if other garments or the machine themselves' might be viable DNA sources of evidence. However, few studies have been conducted on the propensity of DNA to be transferred between garments during laundering.

Blood spatter analysis can help indicate a previous injury by examining the pattern and volume of blood, including the possible transfer of blood from another object. This pattern recognition is typically done on unlaundered clothing and other inanimate objects. Since the pattern (primary or secondary transfer) of the deposit and dispersal of the blood in machine washing is unknown, a chemical could be utilized to indicate the blood's location.

To find evidence investigators use preliminary testing, such as Luminiol, which helps distinguish soils as blood by reacting with the iron found in the hemoglobin within Erythrocytes. If the Luminol reacts in patches on the laundered clothing, it could be argued that that the blood is transferred via physical contact. If the Luminol reacts evenly

on the laundered clothing, it is plausible that the transfer of blood is due to redistribution of DNA material via the water.

Locard's Principle states that when two objects come into contact, they will have cross-transfer of material even if it is not visible to the naked eye. Evidence of this cross transfer itself cannot be wrong (Kirk, 1953) but the analysis of the results may fail to recognize it or understand its meaning. Using this application of Locard's Principle, transfer would occur if the bloodstain were agitated, as it encounters another surface, either before laundering or during the laundering process.

In this regard, cross-transfer can be labeled using the ordinal numbering system to designate how the transfer occurred. Primary transfer is the process of transfer between two objects. Secondary transfer is the process of transfer between a primary object and a secondary object, with the process repeating itself for higher values in the ordinal numbering system.

Locard's system works well for physical contact; however, this is not applicable for aerosol transfer, which is an additional way to comprehend the traditional understanding of Locard's exchange principle. The concept of aerosol transfer is defined as the depositing of material without an intermediary such as sneezing (Gill, 2014). This could be applied to machine laundering, specifically during the drying phase, as the moisture could be aerosolized and transfer DNA material between garments.

In the instance of the analysis of DNA from blood stained fabric, the investigator follows the principle of transfer to support a possible series of events linking a suspect to a crime. The greater the number of transfers required to explain the presence of an individual's DNA on an object, the less weight the evidence tends to carry due to the increasing risks of possible innocent transfer or cross contamination (Gill, 2014).

Research using materials prepared to simulate innocent transfer has shown spermatozoa can be transferred in machine laundering (Kafarowski, Lyon, & Sloan, 1996). Sperm was deposited on clean 100% cotton panties and was washed with simulated loads in the washing machine (Kafarowski, Lyon, & Sloan, 1996). The panties with the sperm were then viewed under UV light. A 0.4 cm^2 cutting was removed for extraction and staining. On average 1-2 sperm were found on each cutting after washing.

These results imply that the presence of sperm on other garments within the same washing load could be from innocent or secondary transfer in regular machine washing. Kafarowski's et al.'s (1996) work supports the theory that sperm cells could withstand the rigors of laundering and suggests that other types of cells could be transferred to other articles in the wash in a similar manor. However, a consideration should be made that sperm have a hard protein coating that may be the reason that they are able to withstand the machine laundering.

There are two criticisms of Kafarowski et al.'s (1996) results and their interpretation. First, an investigation to determine the effects of different washing cycles was not conducted. For example, water temperature could have affected whether sperm was present. Hot water could have removed more sperm from the fabric since some detergents are more effective at degrading DNA at higher temperatures (Castello, Frances, & Verdu, 2012). Secondly, DNA profiling techniques should have been attempted on the sperm. In court, it would not be acceptable to state that sperm was present without identifying the source of the sperm.

However, as transfer occurs sequentially. progressively smaller amounts of material are transferred subsequently limiting the amount of DNA that can be collected. Scientists should be cautious since DNA is everywhere is our environment from one source or another, such as sloughed epithelial cells or shed hairs. This low level of DNA on substrates is typically referred as background DNA. The smaller the amount of collectable material the higher the likelihood that the background DNA will mask smaller samples (Gill, 2014).

Extracted DNA is not able to indicate from which kind of cell it originated. It is therefore necessary to unbiasedly understand all of the possible sources the DNA could be from, and not assume that it is from one source. Peter Gill suggests using the terms active and passive to distinguish between 'relevant' DNA and background DNA. The

context of DNA is important as without it justice professionals may jump to the conclusion that every DNA profile found at a crime scene is an aspect of a crime (Gill, 2014).

Further research investigated innocent transfer by finding trace DNA on bedding during normal contact (Petricevic, Bright, & Cockerton, 2006). Five people used new fitted cotton bed sheets to sleep on their own bed for one night, and bedding that they had no previous contact with for one night. Even with as little as one night of sleep, previous owners of the mattress and the person sleeping on the sheets were correctly identified (Petricevic, Bright, & Cockerton, 2006). Two out of the five volunteers lived in the same house. Although they did not share sleeping areas, they did acknowledge that their bedding was washed together (Petricevic, Bright, & Cockerton, 2006). Trace DNA evidence from the people living together was collected on both of their sheets (Petricevic, Bright, & Cockerton, 2006) suggesting either primary or secondary transfer happened in the course of the laundering process.

While this study demonstrated that DNA could be recovered from limited contact and from previous loads of laundry, there are two concerns with this study. First, the volunteers reused previously used top sheets that may already have contained old skin cells and sweat. This genetic material could have transferred to the new fitted sheet used for testing. New top and fitted sheets should have been provided to the volunteers.

Secondly, the number and ratio of the volunteers is important. There were five people who participated in the study and only one of them was male (Petricevic, Bright, & Cockerton, 2006). It is unclear whether one gender sheds or sweats more at night. These gender differences may affect DNA recovery. Additionally, it was not mentioned if the females were on their menstrual cycle, as they could have potentially deposited some blood on the sheets. Further research on this area would help understand the persistence of low volumes of DNA in the laundry process.

When testing for DNA on cloth material small samples are cut out, as it would be impractical to test the whole shirt. Different fabric weaves could provide more surface area to a given unit of area. This additional surface area to sample area ratio could allow for more DNA cells to be trapped in a given cutting. For example, the difference surface area between a terry cloth used in towels and a knit weave used in t-shirts are very different.

Another consideration of volume to area ratio is the density of the weave, also known as the thread count. Although the weave is the same, the size of the fibers used can affect the compactness of the fabric as a whole. This increased fiber count could provide more surface area that could trap cells (see Figure 13).

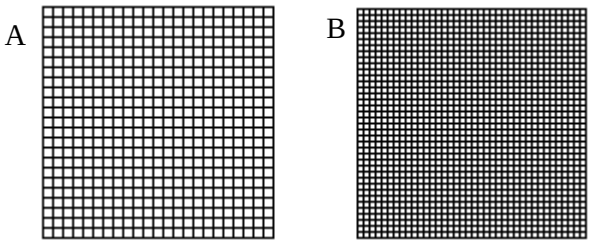

Figure 13: Cartoon example of thread count A) lower thread count which has less threads per square inch B) higher thread count which has a high nuber of threads per inch (Chang, 2016).

Fabric content should be an experimental factor because much of modern fabric is made with synthetic fibers. Non-natural fibers typically have smooth outsides and larger surface to density ratios than natural fibers (Tascan & Edward, 2008) (Figure 14).

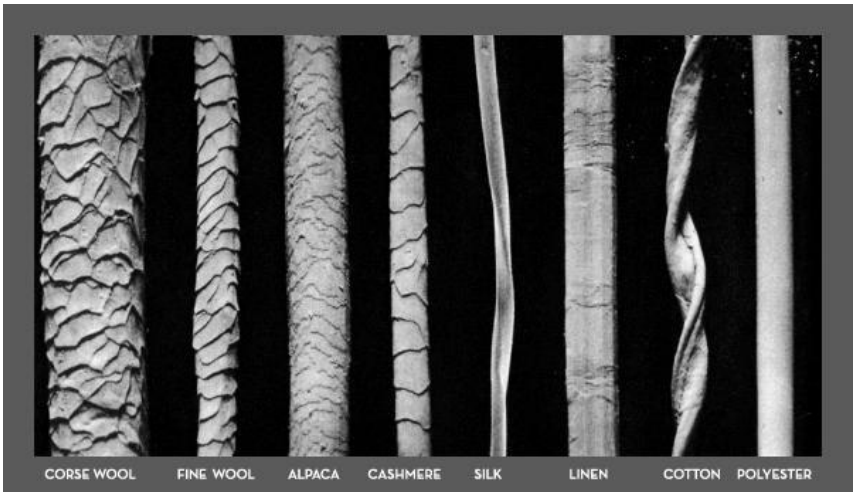

Figure 14: Scanning Electron Microscope depicting the rough exterior of natural and sythetic fibers (Signor, 2016)

However, DNA is more readily removed from natural-fiber fabrics with organic extractions. Cotton is easy to 'digest' and it retains some of the stain while releasing significant amounts of starting material (Goray, Eken, Mitchel, & Van Oorschot, 2009). Cotton and cotton blends are more likely to give positive preliminary results for blood after the fabrics have been washed (Cox, 1990). Future research should be performed on different fabric content so that investigators can make knowledgeable decisions on what fabrics to send out for DNA profiling.

Despite the theoretical capabilities of the retention of DNA in laundered cloth, and evidence of the recovery of human DNA in cloth after laundering, there are very few studies on recovering DNA from laundered bloody clothing. Due to this lack of information, the medical legal community has treated laundered DNA with some skepticism. The doubt over the DNA quality has limited the use of machine laundered bloody clothing as sources of evidence. Laundered evidence exists and the deficiency of information to process it suggests a need to study the effects of laundering DNA evidence and subsequent transfer of DNA material.

According to Locard's principle, there should be DNA transfer within a load and between loads. Studies of sperm and epithelial cells have shown that they are able to withstand machine laundering. These concepts were the basis of the research question of

this study of whether DNA transfers within a primary load and to a secondary or tertiary load? To address this research question four research hypotheses were developed:

1) This is no significant difference between a primary, secondary or tertiary load of laundry on the recovery rate of DNA,
2) A DNA profile is not recoverable from a secondary load,
3) A DNA profile is not recoverable from a tertiary load,
4) For any given laundered item there is no significant difference in the type of laundry item on the recovery rate of DNA.

These research hypotheses will help understand possible scenarios innocent, and otherwise, for the transfer of DNA from blood cells within and between loads in machine laundering. This in turn will help professionals in the forensic science community to effectively implement samples collected from crime scenes as evidence in criminal cases.

METHODS

Sample preparation

Clothing used for this study included: medium sized T-shirt (Gildan 5000), 30x34" denim jeans (Red Kap Men), medium sized Tagless® boxer briefs (Hanes), size 6 women's classic bikini underwear (Hanes), size 6-12 men's tube socks (Hanes) and a 16" x30" 4.5 lb. hand towel (Dynasty Dobby Border). A piece of autoclave tape was folded in with each item inside an autoclave bag and a short piece of autoclave tape was placed on the outside of the bag. The bags were autoclaved with no more than one layer of clothing on the shelf at 120 ^{o}C for 20 minutes and then allowed to come to room temperature. The fabric was stored in the bag until use and was opened in a DNA reduced environment within a biological safety cabinet. If the autoclave tape that was folded with the clothing did not show a positive reaction for sterilization, then the clothing was not used. A "standard load" was comprised of one T-shirt, one set of jeans, one boxer brief, one bikini underwear, one hand towel, and two socks.

Human blood used for this study was obtained from the Community Blood Center/Community Tissue Services (CBC/CTS) in Dayton, Ohio. The blood was donated by volunteers in the Dayton, Ohio area. All donors were given written notice, and consented to the use of their blood in unspecified research. No demographics were released to the researcher at any time, and the researcher did not have any contact with

the donors. A genotype profile was generated from the donated blood for comparison of collected samples (see Table 15).

Table 15: Genotype of Subject 3

Loci	Subject 3
D10S1248	13, 14
vWA	16, 18
D16S539	11, 14
D2S1338	17, 18
Amel	X, Y
D8S1179	13, 14
D21S11	27, 31.2
D18S51	13, 15
D22S1045	16, 16
D19S433	13, 13
TH01	8, 9
FGA	19, 22
D2S441	10, 14
D3S1358	14, 15
D1S1656	17.3, 18.3
D12S391	17, 20

Part One: DNA Material Transfer within a Primary Load

A medium sized T-shirt (Gildan 5000) was used as the substrate for the samples. To prepare the T-shirts, a piece of autoclave tape was folded in with each item inside an autoclave bag, and a short piece of autoclave tape was placed on the outside of the bag. The bags were autoclaved at 120^{o}C for 20 minutes and then allowed to come to room temperature. The fabric was stored in the bag until use and was opened in a DNA reduced environment within a biological safety cabinet. If the autoclave tape that was folded with

the clothing was not showing a positive reaction for sterilization, then the clothing was not used. The prepared fabric or shirts were spread out on a clean sheet of bench paper with the plastic side up in a clear plastic container (see Figure 7) inside a Biological Safety Cabinet (BSC).

The blood was transferred from the donor bag into an autoclaved glass container and stirred. The blood was applied from a height of 10 cm. Five or 10 ml of Subject 3's blood was applied to a prepared T-shirt and washed immediately with a standard load on a 'medium' water level with a 'warm, warm' water temperature on the 'regular' speed setting for 10 minutes. The shirt was transferred to the dryer set to 'normal/perm press' temperature for 50 minutes.

Fabric cuttings (1 cm^2) were cut from the center of each garment and stored in separate 1.5 ml test tubes for extraction (N=21). Each load was stored separately in labeled paper evidence bags

Part Two: DNA Material Transfer between Loads

Immediately after a primary load was removed from the washer a secondary load was added consisting of a standard load without a bloodstained shirt and washed immediately on a 'medium' water level with a 'warm, warm' water temperature on the 'regular' speed setting for 10 minutes. The shirt was transferred to the dryer set to 'normal/perm press' temperature for 50 minutes.

I Immediately after a secondary load was removed from the washer a tertiary load was added consisting of a standard load without a bloodstained shirt and washed immediately on a 'medium' water level with a 'warm, warm' water temperature on the 'regular' speed setting for 10 minutes. The shirt was transferred to the dryer set to 'normal/perm press' temperature for 50 minutes.

A bleach load was used to clean the washer between the tertiary and the primary loads (see Table 16). Fabric cuttings (1 cm^2) were cut from the center of each garment and stored in separate 1.5 ml test tubes for extraction (N=36). Each load was stored separately in labeled paper evidence bags

Table 16: Load order for testing the transfer of DNA material within and between loads of machine-laundered clothing

Sample preparation	Load order	Sample collection
Primary Load: blood stained shirt with 10ml of blood and a standard load. Secondary load: standard load Tertiary load: standard load	Primary Load Secondary load Tertiary load Bleach load Primary Load Secondary load Tertiary load Bleach load Primary Load Secondary load Tertiary load Bleach load	1 cm^2 cuts from the center of each garment

Extraction

This procedure was adapted from AmpFlSTR Profiler Plus PCR Amplification Kit User's Manual (Applied Biosystems, 2012) and Forensic DNA Analysis: A Laboratory Manual (McClintock, 2008). All samples collected for extraction were combined with 500µl of digest buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, pH 7.5) and 15 µl of 10 mg/ ml Proteinase K (Applied Biosystems, 2012). The solution was vortexed for 15 seconds and incubated between eight hours at 56 ^{o}C. Phenol-Chloroform Isoamyl Alcohol (PCIA) (500µl) was added to each tube and vortexed for 15 seconds and centrifuged at 1500 rpm for five minutes. The aqueous layer then was transferred to a new test tube and the PICA step was repeated two more times. Ethanol (1 ml) was added to each tube and incubated for 30 minutes at 0 ^{o}C. The samples were centrifuged for 15 min at 1500 rpm and the liquids removed. Ethanol (1 ml) was added to each tube and vortexed for 15 seconds. The samples were centrifuged for 15 min at 1500 rpm and the liquids removed. The test tubes were allowed to air dry inside of a biological safety cabinet. 36µl of 1x TE-Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) was added to each sample and stored at 0 ^{o}C (McClintock, 2008).

Quantification

Extracted DNA was quantified using the standard protocol from Applied Biosystems for the Human Quantifier Kit User Manual (Applied Biosystems, 2012). ABI Prism® 7900HT Sequence Detection System (Applied Biosystems, 2001) was used to collect data. Either sample (2 µl) or standard (2 µl) was added to separate wells in a MicroAmp Optical 96-Well Reaction Plate (Life Technologies). Master Mix (23 µl) (10.5 µl Quantifier Human Primer mix, 12.5 µl Quantifier PCR Reaction mix) was added to each well and the plate sealed with optical adhesive cover. Thermal cycling parameters consisted of stage one at 95ºC for 10min, stage two was 95 ºC for 15 seconds then 60 ºC for 1 minute with 40 ºC repeats. The IPC and Quantifier Human detectors were set with a standard curve and the 9600 emulation option on.

DNA standard series was made using a stepwise dilution of Quantifiler™ Human DNA Standard with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0 (McClintock, 2008)) following the manufacturer's recommendations. A series of eight standards were run in duplicate with the concentrations ranging from 0.023 ng/µl to 50 ng/µl.

STR Amplification

Using the quantification data, 10 ng of the samples were transferred and air-dried in PCR tubes for shipment to the University College of London in London, England. Polymerase Chain reaction (PCR) was conducted on DNA extracts using AmpFlSTR®

NGM™ PCR Amplification Kit (Applied Biosystems, 2012). Samples were suspended to a concentration of 0.5-1 ng/µl, with 1µl of each sample added to separate PCR test tubes with 15 µl of Master Mix (10.0 µl AmpFlSTR NGM Master Mix, 5.0 µl AmpFlSTR NGM Primer Set). Thermal cycling parameters consisted of 1 minute at 95 ^{o}C, followed by 29-30 cycles of 20 seconds of denaturation at 95 ^{o}C and 3 minutes of annealing at 59 ^{o}C with a final extension for 10 minutes at 60 ^{o}C.

STR Capillary Electrophoresis

5 or 10 µl of standard or amplified DNA was added to separate wells on a 384 well plate and centrifuged for 5 seconds at 1500 rpm. Samples were analyzed on the Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, 2010). The run parameters consisted for a five second injection period, 3 kV per 5 seconds injection voltage, 15.0 kV run voltage, for 120 min, as a 'standard run', with a capillary length of 50cm.

Data Analysis

Quantification Data from the 7900HT Sequence Detection System was analyzed using Statistical Package for Social Sciences® Version 21.0 (IBM, 2012). Two ANOVA analyses were run on the data with two types of post-hoc analyses to account for the type of data collected. Levene's Test was used to test the equality of the variance in the

standard error of the mean (S.E.M.) of different laundry items in a primary load of laundry. To test if there was a significant difference between load order (primary, secondary or tertiary) or item type a two-way ANOVA was completed.

For all comparisons, an overall alpha = 0.05 level of significance was used to determine if the data sets were significantly different. To account for the assumptions of normality and constant variance needed for a Dunnett's Test, the square root of each datum on the amount of DNA recovered were used for the comparison.

Quantification Data from the 7900HT Sequence Detection System was analyzed using Statistical Package for Social Sciences® Version 21.0 (IBM, 2012). A p-value of 0.05 (a=0.05) or less was considered significant unless otherwise noted.

Capillary electrophoresis data were analyzed using GeneMapper version 4.0 (Applied Biosystems, 2006). Recorded alleles exceeded a minimum threshold of 50 relative florescence units (RFUs). If a minor profile was observed then the minor profile was identified by using the assumption that certain alleles were indicative of the original planned profile and had normal morphology for alleles rather than artifacts.

RESULTS

The highest recovery values of transferred DNA from primary loads were found on towels (15.00 pg/µl ± 11.48) and socks (27.84 pg/µl ± 19.77). The lowest recovery values of DNA were found on jeans (0.15 pg/µl ± 0.15) (see Table 17). The average recovery rate of DNA for all of the items washed in conjunction with a 5 ml blood stained shirt was 5.53 pg/µl ± 2.83 (see Table 17) indicating that 4.59 % of DNA from the originally stained garment transferred to other items in the laundering process. The average recovery of DNA from clothing washed with 10ml blood stained shirt was 9.54 ng/µl ± 3.85, indicating that 7.92 % of DNA from the originally stained shirt was transferred in the laundering process (see Table 17). The p-value was less than 0.001 when testing the equality or error variances (Table 18).

When the different item types were separated there was a significant difference (p=0.032) (see Table 19) in the means of recovered DNA. However, there was no statistical correlation between item type and the amount of blood deposited (p= 0.057) (see Table 19).

Table 17: Recovery values of DNA from items of clothing washed in a primary load including a shirt with either 5ml or 10ml of human blood

Item	Experimental Replicate	Unwashed (ng/µl)	5ml (pg/µl)	10ml (pg/µl)
Blood Stained Shirt	1	114.37	35.11	2.54
	2	138.10	17.21	41.24
	3	108.90	0.17	0.64
	Mean ± S.E.M	120.45 ± 8.96	17.49 ± 10.09	14.81 ± 13.23
	Fraction Retained		14.52	12.29
T-shirt	1	-	3.53	0.02
	2	-	0.00	0.00
	3	-	1.07	0.00
	Mean ± S.E.M	- ± -	1.53 ± 1.05	0.01 ± 0.01
	Fraction Transferred		1.27	
Towel	1	-	49.46	37.54
	2	-	1.17	0.00
	3	-	0.12	7.45
	Mean ± S.E.M	- ± -	16.92 ± 16.27	15.00 ± 11.48
	Fraction Transferred		14.04	12.45
Female Underwear	1	-	0.00	0.11
	2	-	1.25	0.00
	3	-	0.00	13.01
	Mean ± S.E.M	- ± -	0.42 ± 0.42	4.37 ± 4.32
	Fraction Transferred		0.35	3.63
Sock	1	-	0.00	3.42
	2	-	3.86	13.12
	3	-	0.15	66.97
	Mean ± S.E.M	- ± -	1.34 ± 1.26	27.84 ± 19.77
	Fraction Transferred		1.11	23.11
Male Underwear	1	-	0.00	2.32
	2	-	2.31	0.00
	3	-	0.09	11.55
	Mean ± S.E.M	- ± -	0.80 ± 0.76	4.62 ± 3.53
	Fraction Transferred		0.66	3.84
Jeans	1	-	0.00	0.00
	2	-	0.00	0.00
	3	-	0.65	0.45
	Mean ± S.E.M	- ± -	0.22 ± 0.22	0.15 ± 0.15
	Fraction Transferred		0.18	0.12
DNA Recovered from Secondary Items	Mean ± S.E.M	120.45 ± 8.96	5.53 ± 2.83	9.54 ± 3.85
	Fraction Transferred		4.59	7.92

Table 18: Differences in mean (I-J) ± Std. error of recovered DNA from different items in a load of laundry

Item Type (I)	Item Type (J)																							
	Bloody shirt			Shirt			Towel			Female Underwear			Sock			Male Underwear			Jeans					
Bloody Shirt	-			0.094	±	0.03	0.079	±	0.03	0.092	±	0.03	0.080	±	0.03	0.092	±	0.03	0.094	±	0.03			
Shirt	-0.094	±	0.03	-			-0.015	±	0.03	-0.001	±	0.03	-0.010	±	0.03	0.002	±	0.03	0.001	±	0.03			
Towel	-0.079	±	0.03	0.015	±	0.03	-			0.014	±	0.03	0.000	±	0.03	0.013	±	0.03	0.016	±	0.03			
Female Underwear	-0.092	±	0.03	0.001	±	0.03	-0.014	±	0.03	-			-0.010	±	0.03	-0.092	±	0.03	0.002	±	0.03			
Sock	-0.080	±	0.03	0.014	±	0.03	-0.001	±	0.03	0.012	±	0.03	-			0.012	±	0.03	0.014	±	0.03			
Male Underwear	-0.092	±	0.03	-0.002	±	0.03	-0.013	±	0.03	0.092	±	0.03	-0.012	±	0.03	-			0.003	±	0.03			
Jeans	-0.094	±	0.03	-0.007	±	0.03	-0.016	±	0.03	-0.002	±	0.03	-0.014	±	0.03	-0.003	±	0.03	-					

Table 19: Comparison of the means of total amount of DNA recovered between loads with either 5 ml and 10 ml of starting material. [a]
Computed using Alpha = 0.05

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial ETA Squared	Noncent. Parameter	Observed Power [a]
Corrected model	0.081	13	0.006	2.485	0.022	0.545	32.3	0.898
Intercept	0.014	1	0.014	5.696	0.024	0.174	5.696	0.634
Amount	0.003	1	0.003	1.373	0.252	0.048	1.373	0.204
Item Type	0.041	6	0.007	2.761	0.032	0.38	16.565	0.786
Amount * Item type	0.036	6	0.006	2.377	0.057	0.346	14.262	0.712
Error	0.068	27	0.003					
Total	0.163	41						
Corrected Total	0.148	40						

The profiles of the samples of transferred DNA with either 5ml or 10ml of blood were consistent with the profile of Subject 3 (see Table 20, for electropherograms see Figure 24 to Figure 26 in Appendix B). STR profiling from samples taken from towels washed with 5 ml or 10 ml of blood had evidence of allele dropout. The towel in the 5ml load had low peak heights and some stutter artifacts resulting in some off ladder allele markers. The towel washed with a 10ml of blood also showed low peak heights, and some abnormal morphology to the alleles.

Table 20: Autosomal STR profiles from laundered articles after laundering in a primary load including a shirt with either 5ml or 10ml of blood. *some artifacts and unusual peak shape observed for allele position

	5 ml of Blood Deposited Before Washing		10 ml of Blood Deposited Before Washing		
Loci	Original Shirt	Towel	Original Shirt	Towel	Subject 3
D10S1248	13, 14	13, 14	13, 14		13, 14
vWA	16, 18	18 *	16, 18		16, 18
D16S539	11, 14	11, 14	11, 14		11, 14
D2S1338	17, 18		17, 18		17, 18
Amel	X, Y	X, Y	X, Y	X, Y	X, Y
D8S1179	13, 14	13,14, OL*	13, 14	13, 14	13, 14
D21S11	27, 31.2	27, 31.2, OL*	27, 31.2	27, 31.2	27, 31.2
D18S51	13, 15	13, 15	13, 15		13, 15
D22S1045	16, 16	16, 16	16, 16	16	16, 16
D19S433	13, 13	13, 13	13, 13	13	13, 13
TH01	8, 9	8 *	8, 9	9, 10	8, 9
FGA	19, 22	19, 22	19, 22	19, 22	19, 22
D2S441	10, 14	10, 11, 12 14, OL*	10, 14	10, 14	10, 14
D3S1358	14, 15	14, 15 *	14, 15	14, 15	14, 15
D1S1656	17.3, 18.3	18.3 *	17.3, 18.3	17.3, 18.3	17.3, 18.3
D12S391	17, 20	17, 20	17, 20		17, 20

There was a decrease from 9.54 ± 3.85 to 0.03 ± 0.01 in the recovery of DNA from articles between consecutive loads of laundry (see Table 21). Articles in the primary

load had the highest observed recovery rates. There was a significant difference between the loads ($p = 0.048$). There was no significant differences when comparing the laundry item type and the load order (see Table 22).

Table 21: Quantity of DNA recovered (pg/µl) from 100% cotton garments after machine laundering with a soiled T-shirt

Item	Experimental Replicate	Primary Load (pg/µl)	Secondary Load (pg/µl)	Tertiary Load (pg/µl)
Blood Stained Shirt	1	0.64	-	-
	2	41.24	-	-
	3	2.54	-	-
	Mean ± S.E.M	14.81 ± 13.23	- ± -	- ± -
	Fraction Retained	0.0123	-	-
T-shirt	1	0.01	0.00	0.06
	2	0.00	0.48	0.00
	3	0.02	0.00	0.00
	Mean ± S.E.M	0.01 ± 0.01	0.16 ± 0.16	0.02 ± 0.02
	Fraction recovered	0.0000	0.0001	0.0000
Towel	1	7.45	0.00	0.03
	2	0.00	1.59	0.06
	3	37.54	0.00	0.00
	Mean ± S.E.M	15.00 ± 11.48	0.53 ± 0.53	0.03 ± 0.02
	Fraction recovered	0.0125	0.0004	0.0000
Female Underwear	1	13.01	0.55	0.00
	2	0.00	0.00	0.22
	3	0.11	0.23	0.00
	Mean ± S.E.M	4.37 ± 4.32	0.26 ± 0.16	0.07 ± 0.07
	Fraction recovered	0.0036	0.0002	0.0001
Sock	1	66.97	0.76	0.01
	2	13.12	0.00	0.00
	3	3.42	0.00	0.00
	Mean ± S.E.M	27.84 ± 19.77	0.25 ± 0.25	0.00 ± 0.00
	Fraction recovered	0.0231	0.0002	0.0000
Male Underwear	1	11.55	1.58	0.00
	2	0.00	0.37	0.08
	3	2.32	0.00	0.00
	Mean ± S.E.M	4.62 ± 3.53	0.65 ± 0.48	0.03 ± 0.03
	Fraction recovered	0.0038	0.0005	0.0000
Jeans	1	0.45	0.03	0.00
	2	0.00	0.60	0.05
	3	0.00	0.00	0.00
	Mean ± S.E.M	0.15 ± 0.15	0.21 ± 0.20	0.02 ± 0.02
	Fraction recovered	0.0001	0.0002	0.0000
Load Items Combined	Mean ± S.E.M	9.54 ± 3.85	0.34 ± 0.12	0.03 ± 0.01
	Fraction Recovered	0.0079	0.0003	0.0000

Table 22: Comparison between PrimarySsecondary/Tertiary and Item type Quantity of DNA recovered (pg/µl)

Item Type	8Mean ± Standard Deviation of DNA recovered		
	Primary	Secondary	Tertiary
Bloody Shirt	0.0148 ± 0.0229	- ± -	- ± -
Shirt	0.0000 ± 0.0000	0.0002 ± 0.0003	0.0000 ± 0.0000
Towel	0.0150 ± 0.0199	0.0005 ± 0.0009	0.0000 ± 0.0000
Female Underwear	0.0044 ± 0.0075	0.0003 ± 0.0003	0.0001 ± 0.0001
Sock	0.0278 ± 0.0342	0.0003 ± 0.0004	0.0000 ± 0.0000
Male Underwear	0.0046 ± 0.0061	0.0007 ± 0.0008	0.0000 ± 0.0000
Jeans	0.0001 ± 0.0003	0.0002 ± 0.0003	0.0000 ± 0.0000
Total	0.0100 ± 0.0180	0.0003 ± 0.0005	0.0000 ± 0.0001

DISCUSSION

The results for the transfer within a primary load showed that there was enough DNA to profile from several garments within the same load as a contaminated garment. This is consistent with previous reports of sperm and DNA from presumably epithelial cells being transferred between fabrics within the washer (Kafarowski, Lyon, & Sloan, 1996; Petricevic, Bright, & Cockerton, 2006). Using a standardized load (Kafarowski, Lyon, & Sloan, 1996) allowed for realistic transfer within the washer, and allowed for different fabric weaves that might have affected the retention of DNA material. However, no one has studied the transfer of blood in the washing machine.

The two highest values for transfer and retention were the sock and the towel. This unusually high retention might be due to the large surface to volume ratio compared to the underwear and other material. Further research would be needed to confirm that the surface area rather than random chance was the deciding factor. This study also used 80% and above cotton fabric, which is consistent with previous laundry studies (Castello, Frances, & Verdu, 2012; 2010; Castello, Frances, & Corella, 2009; Petricevic, Bright, & Cockerton, 2006). Further study of the comparison between artificial and natural materials could lead to preferential selection of natural or even specifically cotton materials.

Limited research has been done to test the transfer between loads, though; these data show that at least a partial profile can be gathered from an independent load from a bloodstained garment. The method of transfer of genetic material from a primary load to a secondary load is not immediately clear but it could be transfer from water left in the

washing machine or where the clothing come in contact on the inside surfaces. To test this theory water samples before and after each load should be tested.

There are different types of washing machines and washing cycles. This study was limited to one top loading washer with limited settings. Newer models have 'sanitizing' options and other features that were not available for testing. Front loading washing machines also have a different method of agitating the clothing, which might result in different values. Further research should focus on this aspect since they can be frequently found in private homes. Only two volumes of DNA were used as starting material, which limits the statistical value of the data, further research could test larger volumes of starting material.

After reviewing the results, it is clear that some DNA can be recovered from items in a secondary load. However, the data does not statistically support the hypothesis that DNA can be transferred from a primary to a tertiary load (see Table 21). Therefore, the obtained DNA values that showed transfer from the primary to a tertiary load were by pure chance.

After analyzing the amounts recovered by the garment type it is clear that the towel and socks are the best sources for recovering transferred DNA material within the load (see Table 21). These data graphically appears to have sizeable difference between item type; however mathematically they are not sufficiently different. Since the p-value was so small, it indicated that there was a large inequality between the error variances. This inequality could be the cause of the disparity between the statistical analysis and the raw data appearances.

This study is limited due to the used of sterilized equipment and clothing and does not take into account the variability of dirty clothing like sweat. The next research question could do the same thing with epithelial cells and blood so that not only a victim can be identified but the perpetrator. The data from this study would help investigators establish the veracity of recovered evidence. For instance, if bloodstains are found on a child's garment, innocent secondary transfer within the washer can be included or excluded by analyzing other appropriate items such as towels and socks collected from the same wash load.

Although the rate of recovery is small compared to the amounts deposited, this information can help investigators prioritize samples that might have higher amounts of DNA per square centimeter. All of the samples that were genotyped were consistent with the DNA material donor. Although some over amplification was observed, the high fidelity of the profiles indicates this procedure could be utilized for criminal investigations.

CONCLUSIONS

Despite current ambivalence to using laundered clothing as a source of DNA, this study demonstrates the ability to recover DNA from machine laundered, bloodstained clothing. There are still many factors such as substrate material, effect of high efficient washers, and multiple DNA sources that need to be examined. However, the data supported that human DNA is recoverable from a primary load and from most items within the wash. The most likely source of transfer DNA was towels and socks due to the large surface to volume ratio. Secondary transfer of DNA was observed to a secondary load of laundry. Unfortunately, although traces of DNA were detected from the tertiary load of laundry it was not enough for DNA profiling. This information is vital to understanding the nuances of DNA that has been exposed to machine laundering within the field of forensic science and could lead to better evaluation of crime scene evidence.

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APPENDIX A

Table 23: List of ingredients for 18 detergents used to see the effect of laundering additives on blood.

Manufacturers Label	List Of Ingredients
All Stainlifters Free & Clear	Water, Sodium Dodecylbenzenesulfonate, Ethoxylated Lauryl Alcohol, Sodium Silicate, Sodium Hydroxide, Coconut Fatty Acid Salt, Sodium Xylenesulfonate, Stilbene Disulfonic Acid Triazine Derivative,
All Oxi-Active Stainlifter	Water, Ethoxylated Lauryl Alcohol, Sodium Hydroxide, Citric Acid, Methyl Ester Sulfonate, Sodium Dodecylbenzenesulfonate, Triethanolamine, Protease, Tetrasodium Iminodisuccinate, Modified Polycarboxylate, Coconut Fatty Acid Salt, Perfume, Stilbene Disulfonic Acid Triazine Derivative, Benzisothiazolinone, Methylisothiazolinone, Amylase, Calcium Chloride, Methylchloroisothiazolinone, Methylisothiazolinone, Acid Blue 80, Enzyme
Ultra Ajax With Bleach Alternative	Water, C14-15 Pareth 7, Taurus 134, Sodium Carbonate, Sodium Laureth Sulfate, Dye, Fragrance, 4preservative, Lauramidopropylamine Oxide, Optiblanc Nl, Sodium Bicarbonate
Downy	Water, Diethyl Ester Dimethyl Ammonium Chloride, Fragrance, Starch, Ammonium Chloride, Calcium Chloride, Formic Acid, Polydimethylsiloxane, Liquitint™ Blue, Diethylenetriamine Pentaacetate, Sodium Salt
Gain Original Fresh Lock	Water, Alcoholethoxy Sulfate, Borax, Linear Alkylbenzene Sulfonate, Ethanolamine, Citric Acid, Diethylene Glycol, Propylene Glycol, Polyethyleneimine Ethoxylate, Dtpa, Lauramine Oxide, Alcohol Ethoxylate, Isodium Diaminostilbene Disulfonate, Diquaternium Ethoxysulfate, Sodium Formate, Calcium Formate, Protease, Liquitint™ Green, Amylase, Dipropylethyl Tetramine, Dimethicone, Hydrogenated Castor Oil, Fragrance
Clorox Green Works Oxi Stain Remover	Sodium Carbonate, Sodium Percarbonate

Manufacturers Label	List Of Ingredients
Method Laundry Detergent	Coco/Soy Methyl Ester, Lauryl And Oleoyl Alcohol Ethoxylates, Glycerin, Sodium Alkane Sulfonate, Peg 300 Monooctyl Either, Decyl Glucoside, Ethyl Levulinate Glycerol Ketal, Carboxymethylinulin, Mipa-Lactate, Cellulose, Protease, Amylase, Mannanase, Lipase, Ethanol, Purified Water, Fragrance Oil Blend , Carboxylate Polymer, Distyrylbiphenolsulfonate, Propane Diol, Sulfonate / Ethoxylate
Seventh Generation Natural 2x Concentrated Laundry Detergent,	Water, Sodium Lauryl Sulfate, Laureth-6, Sodium Citrate, Glycerin, Boric Acid, Sodium Chloride, Oleic Acid, Sodium Hydroxide, Calcium Chloride, Protease, Amylase, Mannanase, Methylisothiazolinone (And) Benzisothiazolinone, Citric Acid
Tide Free & Gentle	Water, Sodium Alcoholetoxy Sulfate, Propylene Glycol, Borax, Ethanol, Linear Alkylbenzene Sulfonate Sodium Salt, Polyethyleneimine Ethoxylate, Diethylene Glycol, Trans Sulfated & Ethoxylated Hexamethylene Diamine, Alcohol Ethoxylate, Linear Alkylbenzene Sulfonate, Mea Salt, Sodium Formate, Sodium Alkyl Sulfate, Dtpa, Amine Oxide, Calcium Formate, Disodium Diaminostilbene Disulfonate, Amylase, Protease, Dimethicone,
Ultra Tide Vivid White + Bright Plus Bleach Original	Sodium Carbonate, Sodium Aluminosilicate, Sodium Sulfate, Linear Alkylbenzene Sulfonate Sodium Percarbonate, Nonanoyloxybenzenesulfonate, Alkyl Sulfate, Water, Silicate, Sodium Polyacrylate, Ethoxylate, Polyethylene Glycol 4000, Fragrance Dtpa Palmitic Acid, Protease, Disodium Diaminostilbene Disulfonate, Silicone, Fd&C Blue 1, Cellulase, Alkyl Either Sulfate
Tide Original Scent	Water, Alcoholethoxy Sulfate, Linear Alkylbenzene Sulfonate, Propylene Glycol, Citric Acid, Sodium Hydroxide, Borax, Ethanolamine, Ethanol, Alcohol Sulfate, Polyethyleneimine Ethoxylate, Sodium Fatty Acids, Diquaternium Ethoxysulfate, Protease, Diethylene Glycol, Laureth-9, Alkyldimethylamine Oxide, Fragrance, Amylase, Disodium Diaminostilbene Disulfonate, Dtpa, Sodium Formate, Calcium Formate, Polyethylene Glycol 4000, Mannanase, Liquitint™ Blue, Dimethicone

Manufacturers Label	List Of Ingredients
Tide Pods Spring Meadow	Water, Linear Alkylbenzene Sulfonates, C12-16 Pareth-9, Propylene Glycol, Alcoholethoxy Sulfate, Polyethyleneimine Ethoxylate, Glycerine, Fatty Acid Salts, Polyvinyl Alcohol Film, Peg-136 Polyvinyl Acetate, Ethylene Diamine Disuccinic Salt, Monoethanolamine Citrate, Sodium Bisulfite, Diethylenetriamine Pentaacetate, Sodium, Disodium Distyrylbiphenyl Disulfonate, Calcium Formate, Mannanase, Xyloglucanase, Sodium Formate, Hydrogenated Castor Oil, Natalase, Dyes, Termamyl, Subtilisin, Benzisothiazolin, Perfume,
Wisk Deep Clean Original	Water, Alcohol Either Sulfate, C12-15 Pareth-7, Sodium Dodecylbenzenesulfonate, Sodium Hydroxide, Methyl Ester Sulfonate, Citric Acid, Triethanolamine, Modified Polycarboxylate, Protease, Tetrasodium Iminodisuccinate, Coconut Fatty Acid Salt, Perfume, Amylase, Disodium Distyrylbiphenyl Disulfonate, Benzisothiazolinone, Methylisothiazolinone, Methylchloroisothiazolinone, Methylisothiazolinone, Acid Blue 80, Enzyme
Bounce Outdoor Freshness	Dipalmethyl Hydroxyethylammoinum Methosulfate, Fatty Acid, Polyester Substrate, Clay, Fragrance
Tide To Go, Deionized Water	Dipropylene Glycol Butyl Either, Sodium Alkyl Sulfate, Hydrogen Peroxide, Ethanol, Magnesium Sulfate, Alkyl Dimethyl Amine Oxide, Citric Acid, Sodium Hydroxide, Trimethoxy Benzoic Acid, Fragrance
Oxi Clean Max Force	Water, Undeceth-5, Propylene Glycol, Dihydoxyethyl Tallow Glycinate, Acrylic Acid Homopolymer, Dipropylene Glycol Butyl Either, Sodium Tetraborate Decahydrate, Sodium Carbonate, Fragrance, Proteolytic Enzyme, Sodium Citrate, Acrylic Polymer, Quaternium-15, Alpha-Amylase Enzyme, Calcium Chloride
New Ultra Ajax With Stain Fighter & Color Booster	Water, C14-15 Pareth 7, Taurus 134, Sodium Carbonate, Sodium Laureth Sulfate, Dye, Fragrance, Preservative, Lauramidopropylamine Oxide, Optiblanc Nl, Sodium Bicarbonate

Manufacturers Label	List Of Ingredients
Seventh Generation Natural Laundry Detergent (Powder)	Sodium Carbonate, Laureth-6, Sodium Citrate, Sodium Silicate, Sodium Aluminosilicate, Sodium Bicarbonate, Sodium Percabonate, Magnesium Sulfate, Cocos Nucifera Oil, Lauyl Polyglucose, Cellulose Cum, Sodium Carboxymethyl Inulin, Protease, Amylase

Figure 15: Image of washer and dryer used for this study

Table 24: Recorded water temperature for washer used for the study

Washer setting (Wash, Rinse)	Wash cycle 1	Wash cycle 2	Wash cycle 3	Mean (C^o)	Rinse Cycle 1	Rinse Cycle 2	Rinse Cycle 3	Mean (C^o)
Warm, Warm	26.9	26.9	28.9	27.57	29.2	28.2	28.5	28.6
Warm, Cold	29.3	28.3	28.9	28.83	13.7	13.5	13.9	13.7
Cold, Cold	12.7	12.1	12.3	12.37	12.2	12.3	12.5	12.3
Hot, Cold	47.7	44.8	48.5	47	13.3	13.1	13.2	13.2

Table 25: Recorded amount of water used by the Kenmore three speed with speed control automatic washer

Programed Size	Amount of water (ml)	Amount of water (ml)	Amount of water (ml)	Mean (ml):
Large	83820.33	89225.9	87874.51	86973.58
Medium	67709.63	71654.03	70298.85	69887.5
Small	45966.23	47279.77	46776.31	46674.11

Table 26: Dunnett's Test results for multi to one comparison of water to undiluted laundry additives. *Comparisons are significant at the 0.05 level

	Additive Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
Positive control	all® 2X Ultra Free Clear Laundry Detergent, 50 oz.	-8.752*	-13.621	-3.883
Positive control	all® HE 2x Ultra Oxi-Active® Stainlifters™ Laundry Detergent, Waterfall Clean, 50 oz.	-3.500	-8.369	1.369
Positive control	Ajax® 2x Ultra Liquid Laundry Detergent with Bleach Alternative, 50 oz.	-6.183*	-11.052	-1.314
Positive control	Downy® Non Concentrated Fabric Softener, April Fresh, 64 oz.	-10.951*	-15.820	-6.082
Positive control	Ultra Gain® 2x Liquid Laundry Detergent, Original , 50 oz.	-9.757*	-14.626	-4.888
Positive control	Clorox® Green Works™ Oxi Stain Remover, Unscented, 56 oz. Container	-11.041*	-15.910	-6.172
Positive control	Method® HE Laundry Detergent, Free And Clear, 20 oz.	-9.800*	-14.669	-4.931
Positive control	Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.	-3.326	-8.195	1.543
Positive control	Tide® Free & Gentle 2x Concentrated Laundry Detergent, 100 fl. Oz	-6.223*	-11.092	-1.354

	Additive Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
Positive control	Tide® Powder Laundry Detergent with Bleach, 144 oz.	-11.041*	-15.910	-6.172
Positive control	Tide® HE Laundry Detergent, Original Scent, 100 oz.	-7.013*	-11.882	-2.144
Positive control	Tide® PODS™ Spring Meadow, 72 Ct.	-7.069*	-11.938	-2.200
Positive control	Wisk® 2X Liquid Detergent, Deep Clean, 50 fl oz	-10.932*	-15.801	-6.063
Positive control	Bounce® Fabric Softener Sheet, Outdoor Fresh	-9.547*	-14.416	-4.678
Positive control	Tide® To Go Pen	-7.837*	-12.706	-2.968
Positive control	OxiClean® Max Force® Laundry Stain Remover Spray, 12 oz.	-7.571*	-12.440	-2.702
Positive control	Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, 50 oz.	-5.841*	-10.710	-0.972
Positive control	Seventh Generation® Free & Clear Natural Laundry Detergent, Unscented, 112 oz. Box	-11.041*	-15.910	-6.172

Table 27: Dunnett's Test results for multi to one comparison of water to diluted laundry additives. *Comparisons are significant at the 0.05 level

	Additive Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
Positive control	all® 2X Ultra Free Clear Laundry Detergent, 50 oz.	-8.5727*	-11.4057	-5.7397
Positive control	all® HE 2x Ultra Oxi-Active® Stainlifters™ Laundry Detergent, Waterfall Clean, 50 oz.	-8.8523*	-11.6853	-6.0193
Positive control	Ajax® 2x Ultra Liquid Laundry Detergent with Bleach Alternative, 50 oz.	-10.7345*	-13.5675	-7.9015
Positive control	Downy® Non Concentrated Fabric Softener, April Fresh, 64 oz.	-10.6959*	-13.5289	-7.8629
Positive control	Ultra Gain® 2x Liquid Laundry Detergent, Original , 50 oz.	-10.6868*	-13.5198	-7.8538
Positive control	Clorox® Green Works™ Oxi Stain Remover, Unscented, 56 oz. Container	-10.2796*	-13.1126	-7.4466
Positive control	Method® HE Laundry Detergent, Free And Clear, 20 oz.	-9.6222*	-12.4552	-6.7892
Positive control	Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.	-9.0649*	-11.8979	-6.2319
Positive control	Tide® Free & Gentle 2x Concentrated Laundry Detergent, 100 fl. Oz	-10.5169*	-13.3499	-7.6839

	Additive Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
Positive control	Tide® Powder Laundry Detergent with Bleach, 144 oz.	-10.5996*	-13.4325	-7.7666
Positive control	Tide® HE Laundry Detergent, Original Scent, 100 oz.	-10.8218*	-13.6547	-7.9888
Positive control	Tide® PODS™ Spring Meadow, 72 Ct.	-9.0080*	-11.8410	-6.1750
Positive control	Wisk® 2X Liquid Detergent, Deep Clean, 50 fl oz	-9.9841*	-12.8171	-7.1511
Positive control	Bounce® Fabric Softener Sheet, Outdoor Fresh	-10.4641*	-13.2971	-7.6311
Positive control	Tide® To Go Pen	-10.4641*	-13.2971	-7.6311
Positive control	OxiClean® Max Force® Laundry Stain Remover Spray, 12 oz.	-9.5389*	-12.3719	-6.7059
Positive control	Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, 50 oz.	-8.2380*	-11.0710	-5.4050
Positive control	Seventh Generation® Free & Clear Natural Laundry Detergent, Unscented, 112 oz. Box	-9.9680*	-13.1354	-6.8007

Table 28: ANOVA results with Bonferroni correction comparing undiluted results to diluted results for each laundry addditive independently

Manufacturer's Label	p-value
all® 2X Ultra Free Clear Laundry Detergent, 50 oz.	0.6196
all® HE 2x Ultra Oxi-Active® Stainlifters™ Laundry Detergent, Waterfall Clean, 50 oz.	0.0008[b]
Ajax® 2x Ultra Liquid Laundry Detergent with Bleach Alternative, 50 oz.	0.0013[b]
Downy® Non Concentrated Fabric Softener, April Fresh, 64 oz.	0.0399
Ultra Gain® 2x Liquid Laundry Detergent, Original, 50 oz.	0.1233
Clorox® Green Works™ Oxi Stain Remover, Unscented, 56 oz. Container	0.0199
Method® HE Laundry Detergent, Free And Clear, 20 oz.	0.5965
Seventh Generation® Natural 2X Concentrated Laundry Detergent, 50 oz.	0.1162
Tide® Free & Gentle 2x Concentrated Laundry Detergent, 100 fl. Oz	0.1313
Tide® Powder Laundry Detergent with Bleach, 144 oz.	0.4239
Tide® HE Laundry Detergent, Original Scent, 100 oz.	0.0111
Tide® PODS™ Spring Meadow, 72 Ct.	0.1996
Wisk® 2X Liquid Detergent, Deep Clean, 50 fl oz	0.0128
Bounce® Fabric Softener Sheet, Outdoor Fresh	0.4525
Tide® To Go Pen	0.0599
OxiClean® Max Force® Laundry Stain Remover Spray, 12 oz.	0.0013[b]
Ajax® 2x Ultra HE Liquid Laundry Detergent, Original Scent, 50 oz.	0.3762
Seventh Generation® Free & Clear Natural Laundry Detergent, Unscented, 112 oz. Box	0.4226

Table 29: Recovered amounts of DNA from substrates prepared for experimentation as a control study.

Sample Source	Experimental replicate	Technical replicate 1	2	3	Mean	±	S.E.M
Aluminum foil Wrapped cloth	1	0.000	0.000	0.000	0.00	±	0.00
	2	0.000	0.000	0.000	0.00	±	0.00
	3	0.000	0.000	0.000	0.00	±	0.00
T-shirt	1	0.000	0.000	0.000	0.00	±	0.00
	2	0.000	0.000	0.000	0.00	±	0.00
	3	0.000	0.000	0.000	0.00	±	0.00
Towel	1	0.000	0.000	0.000	0.00	±	0.00
	2	0.000	0.000	0.000	0.00	±	0.00
	3	0.000	0.000	0.000	0.00	±	0.00
Jeans	1	0.000	0.000	0.000	0.00	±	0.00
	2	0.000	0.000	0.000	0.00	±	0.00
	3	0.000	0.000	0.000	0.00	±	0.00

Table 30: ANOVA comparison of the differences of total amount of DNA recovered between loads with either 5ml and 10ml of starting material. [a] computed using alpha =.05

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial ETA Squared	Noncent. Parameter	Observed Power [a]
Corrected model	0.081	13	0.006	2.485	0.022	0.545	32.3	0.898
Intercept	0.014	1	0.014	5.696	0.024	0.174	5.696	0.634
Amount	0.003	1	0.003	1.373	0.252	0.048	1.373	0.204
Item Type	0.041	6	0.007	2.761	0.032	0.38	16.565	0.786
Amount * Item type	0.036	6	0.006	2.377	0.057	0.346	14.262	0.712
Error	0.068	27	0.003					
Total	0.163	41						
Corrected Total	0.148	40						

APPENDIX B

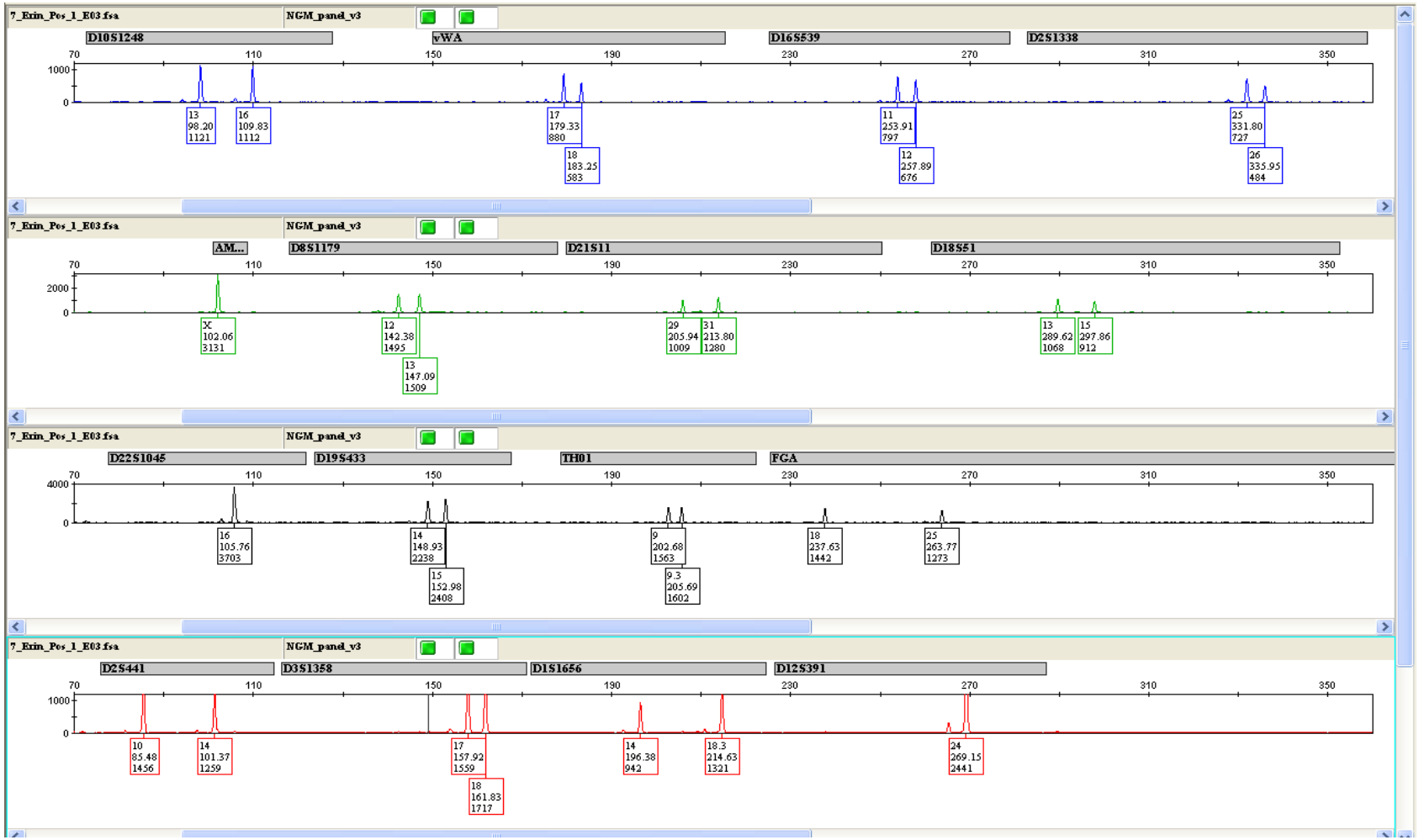

Figure 16: Subject 1’s reference DNA profile

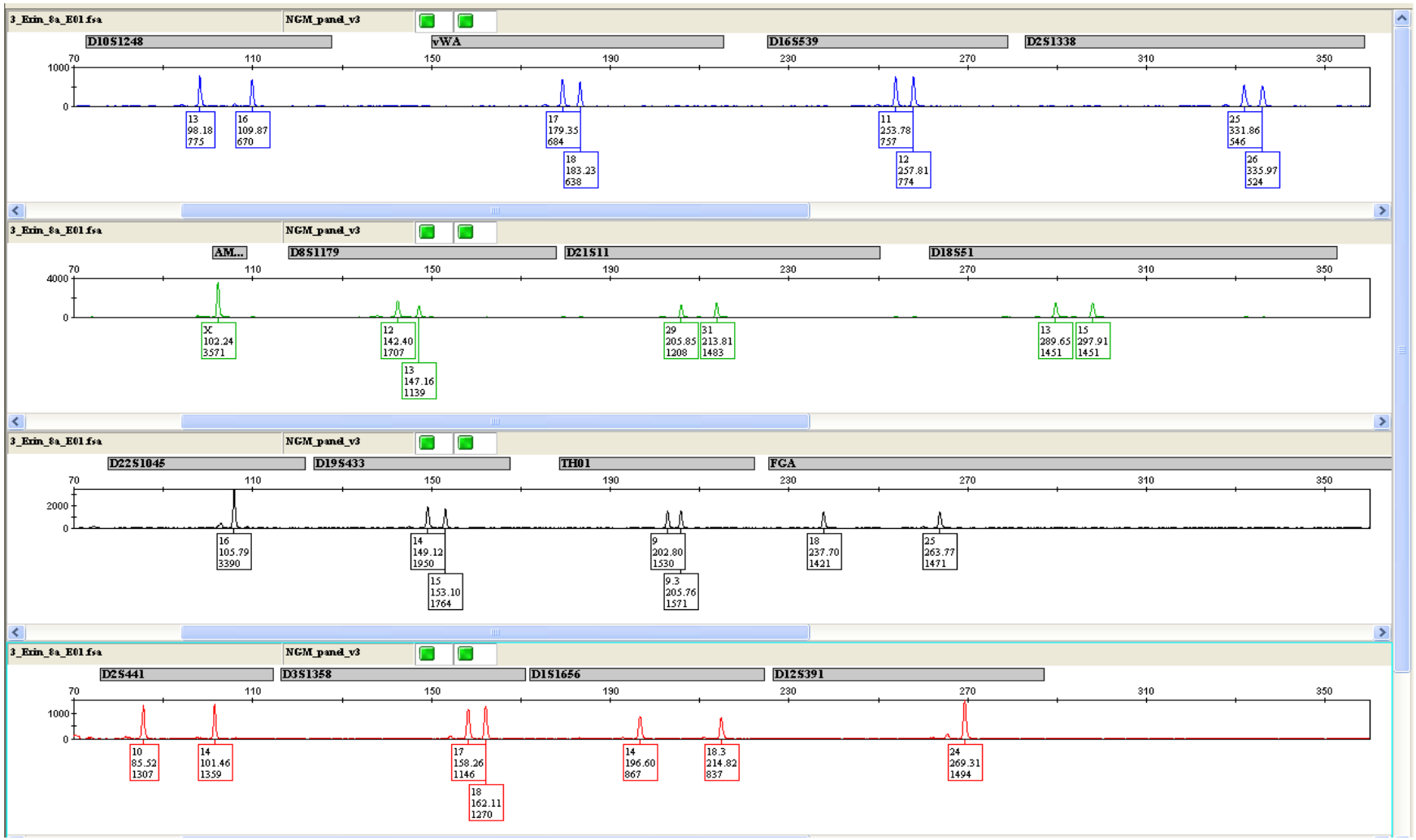

Figure 17: Electropherogram of undiluted laundry additive 8 with blood.

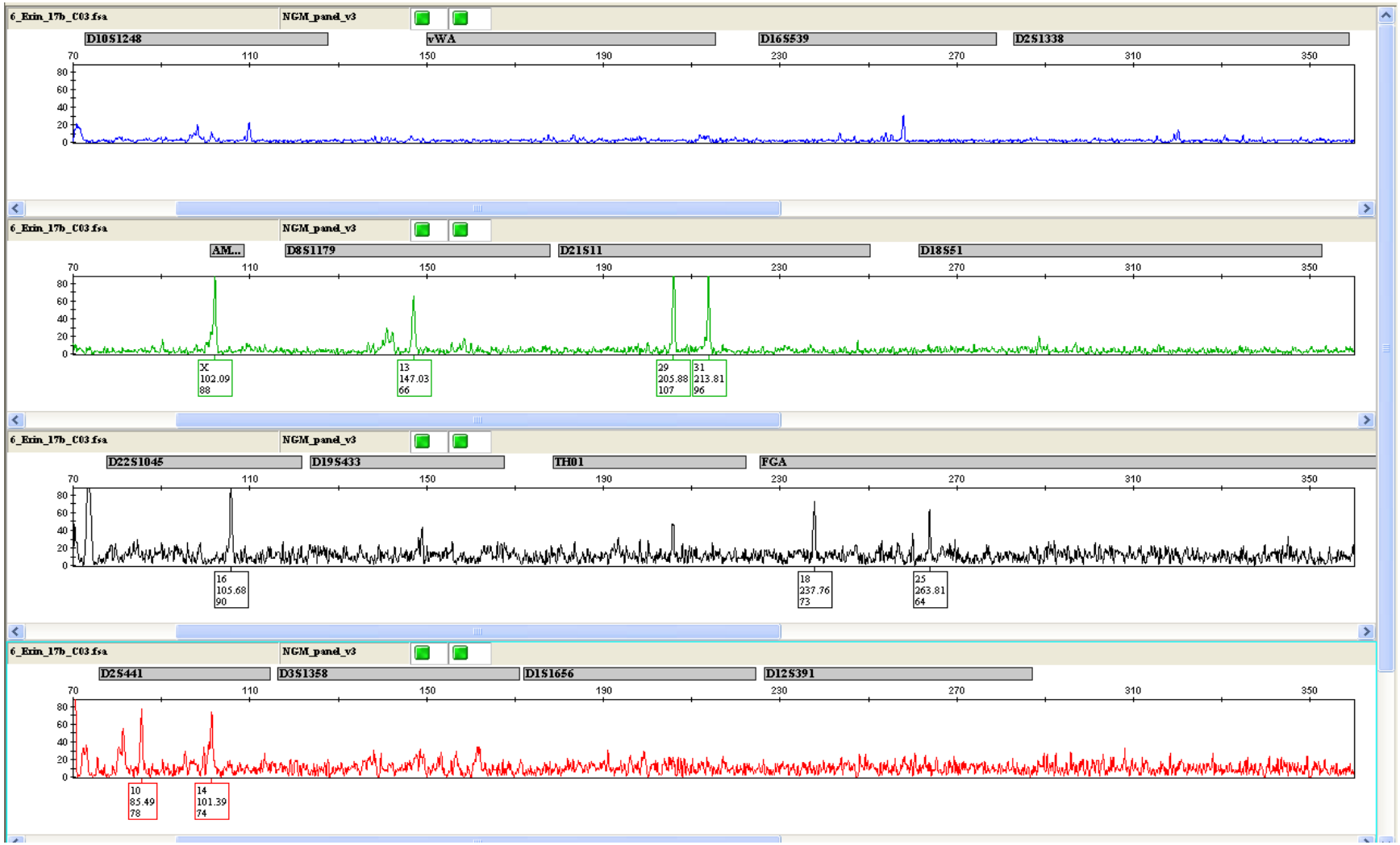

Figure 18: Electropherogram of undiluted laundry additive 17 with blood.

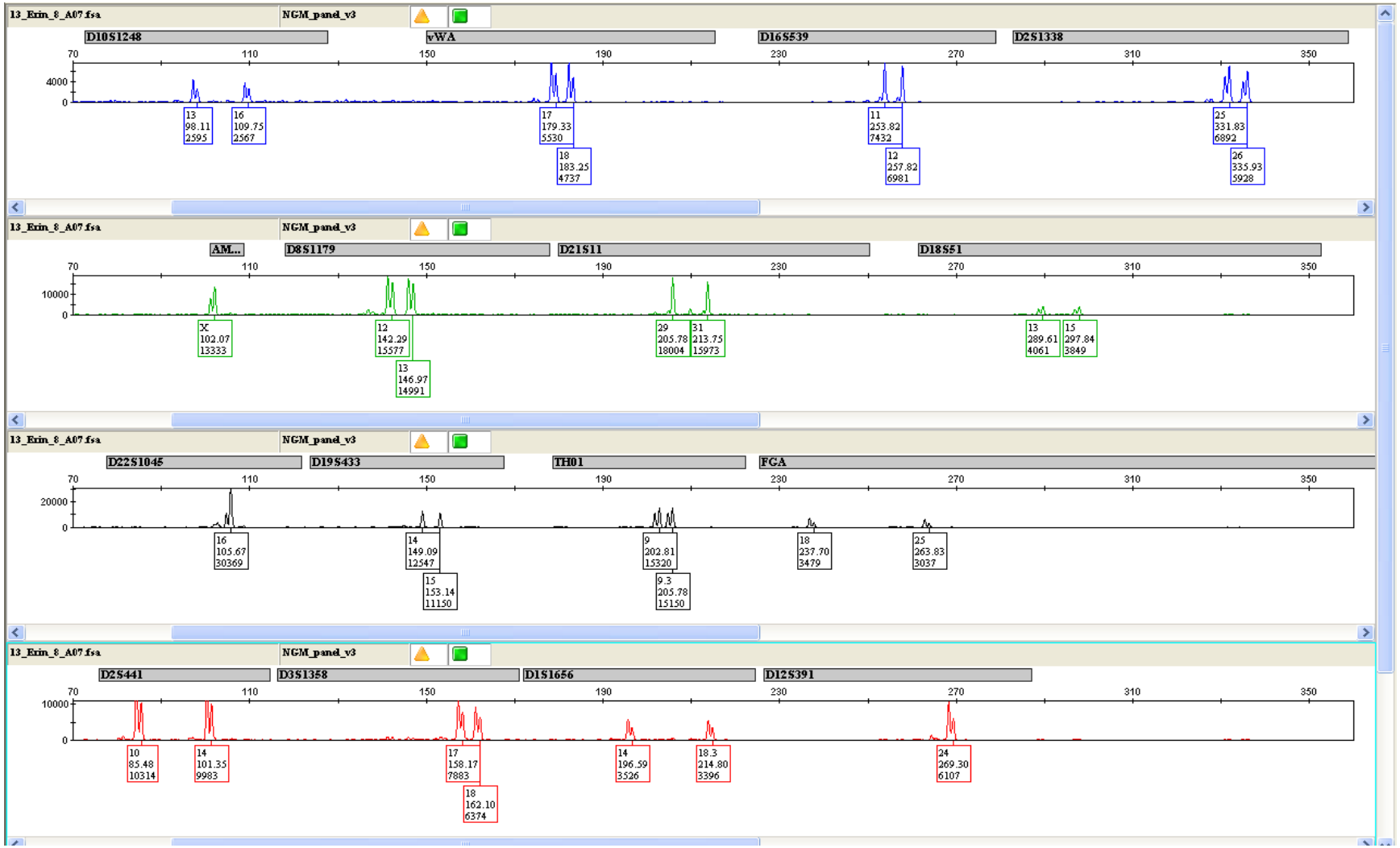

Figure 19: Electropherogram of diluted laundry additive 8 with blood.

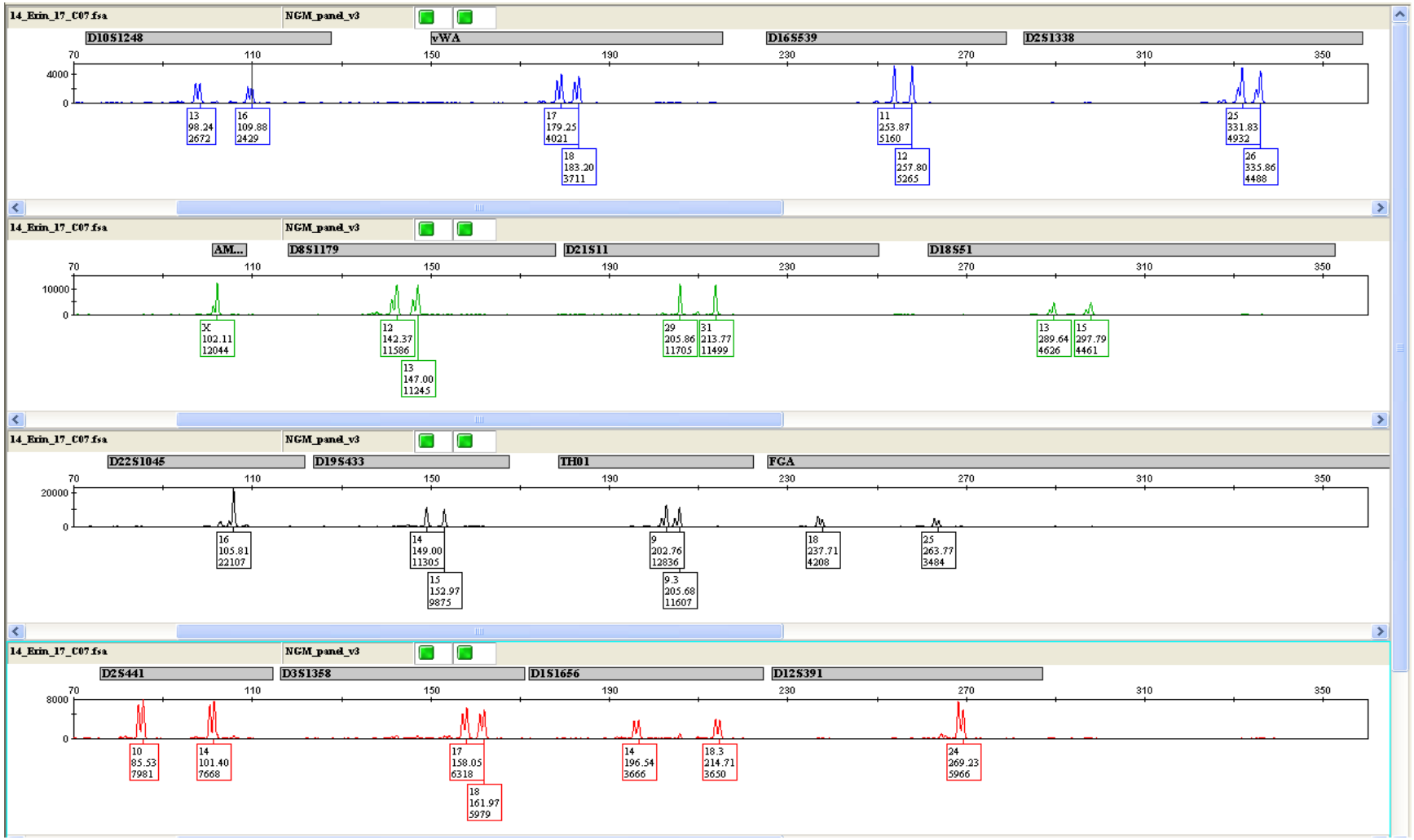

Figure 20: Electropherogram of diluted laundry additive 17 with blood.

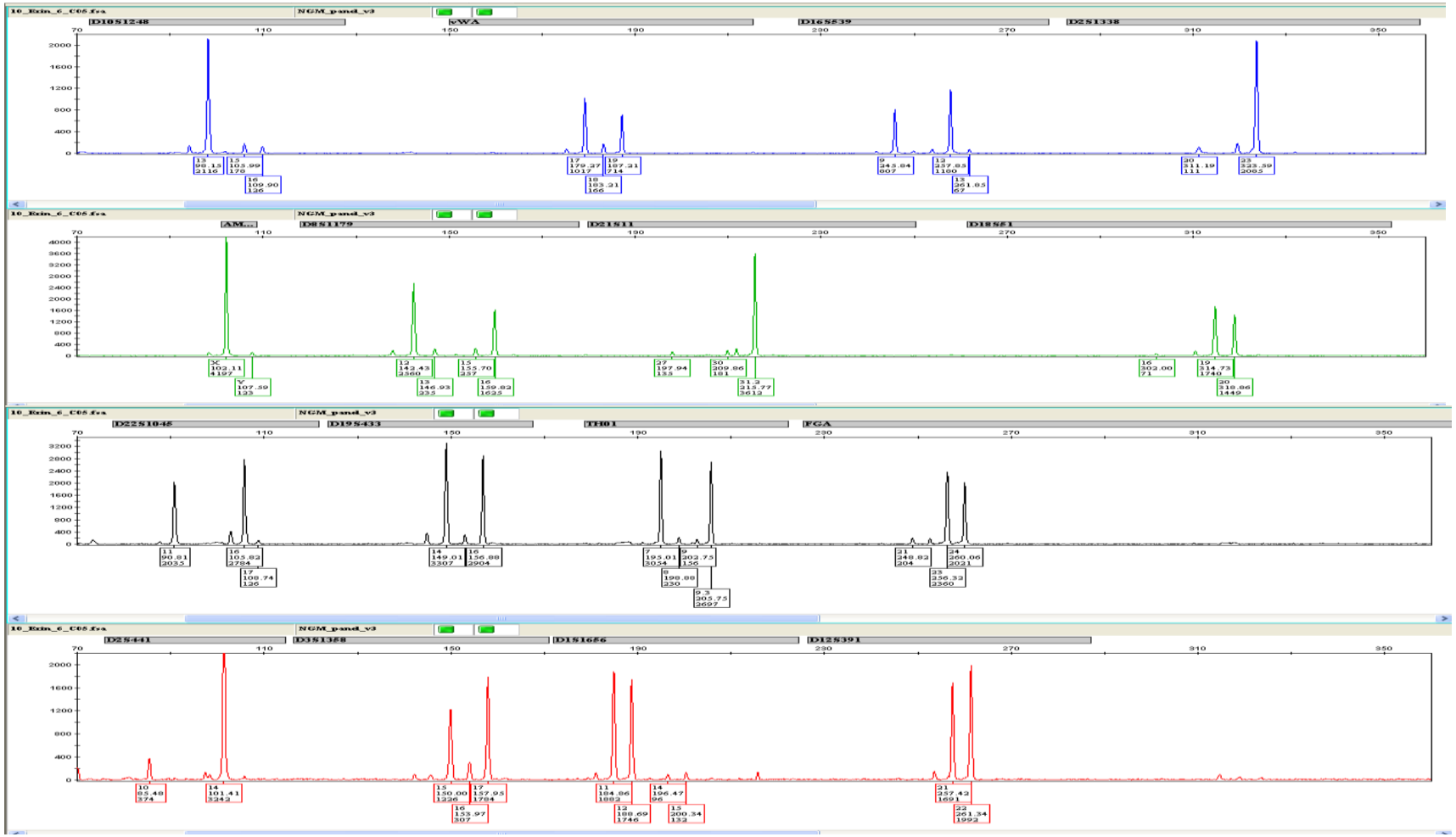

Figure 21: Electropherogram from T-Shirt with immediate washing.

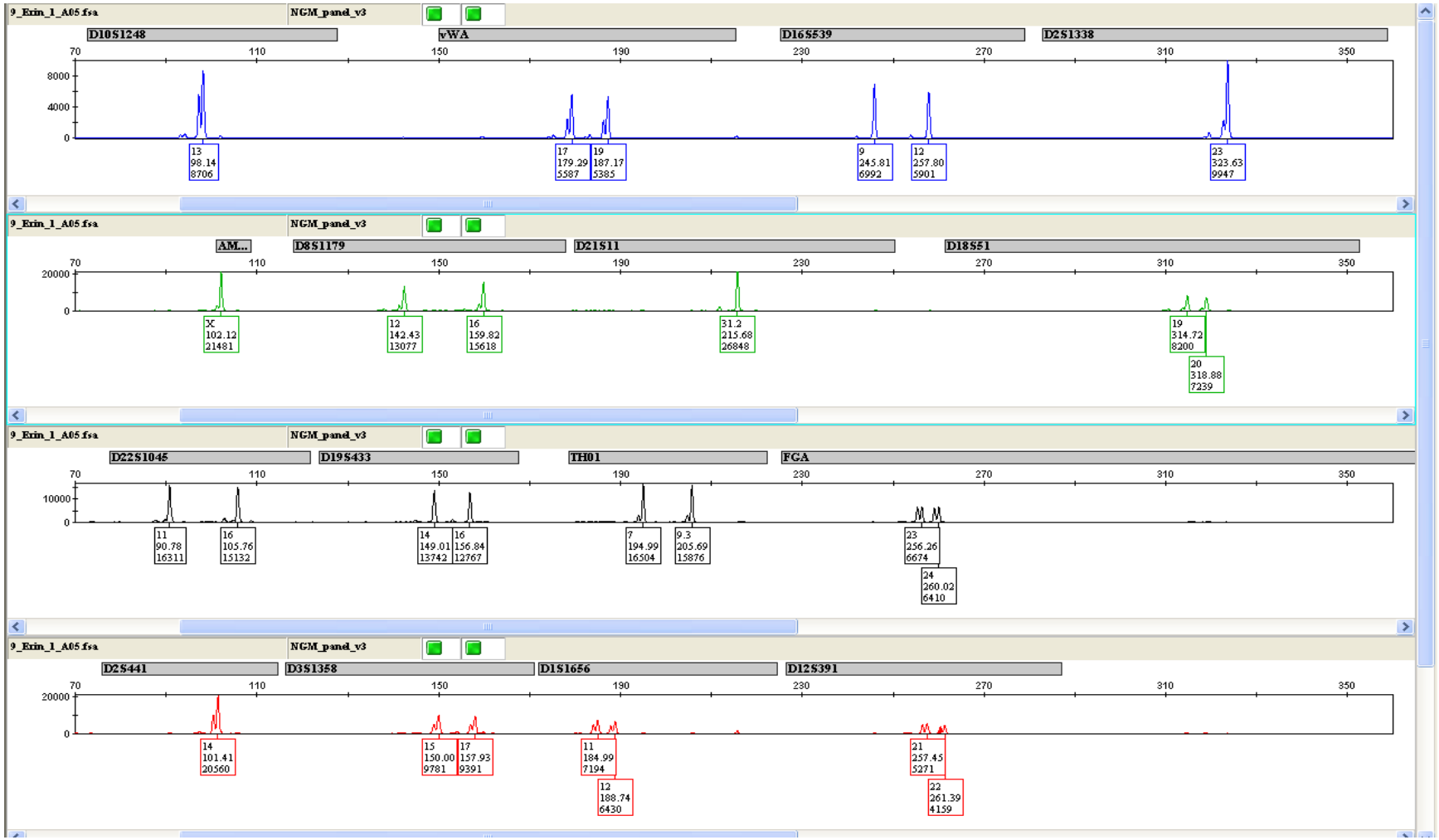

Figure 22: Electropherogram from T-shirt with one-hour delay before washing.

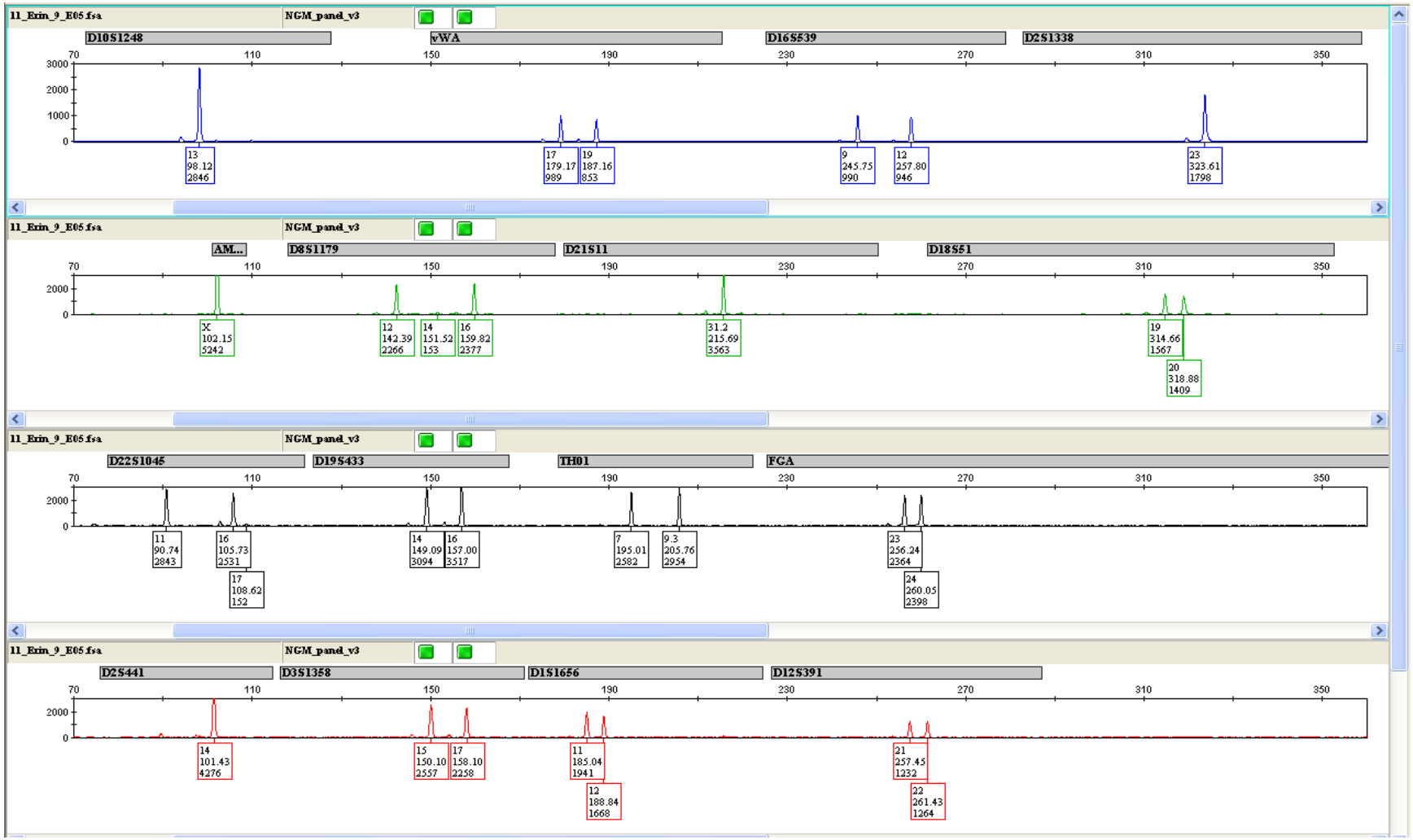

Figure 23: Electropherogram from T-shirt with a twenty-four hour delay before washing.

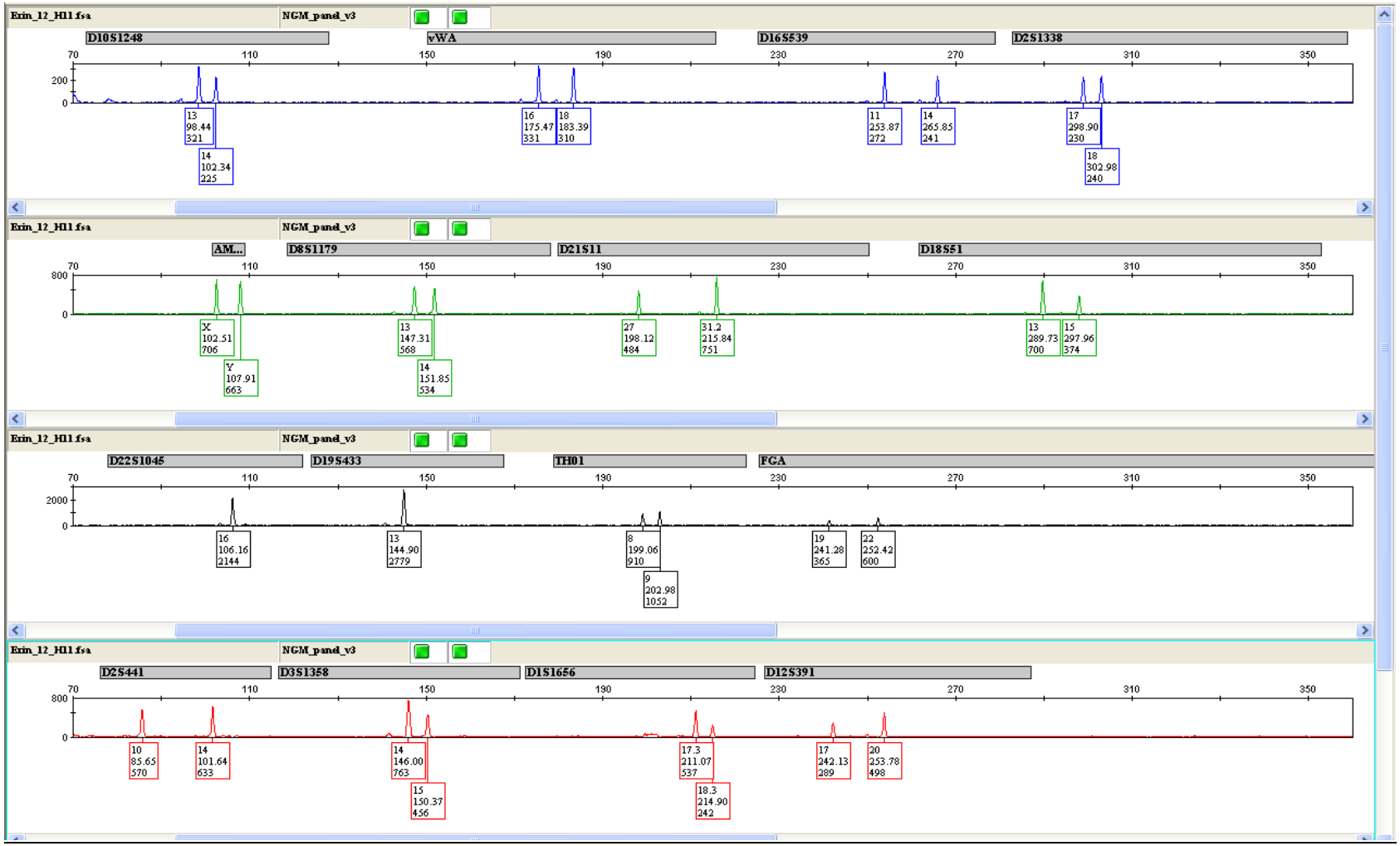

Figure 24: Electropherogram from laundered T-shirt with 5 ml of blood.

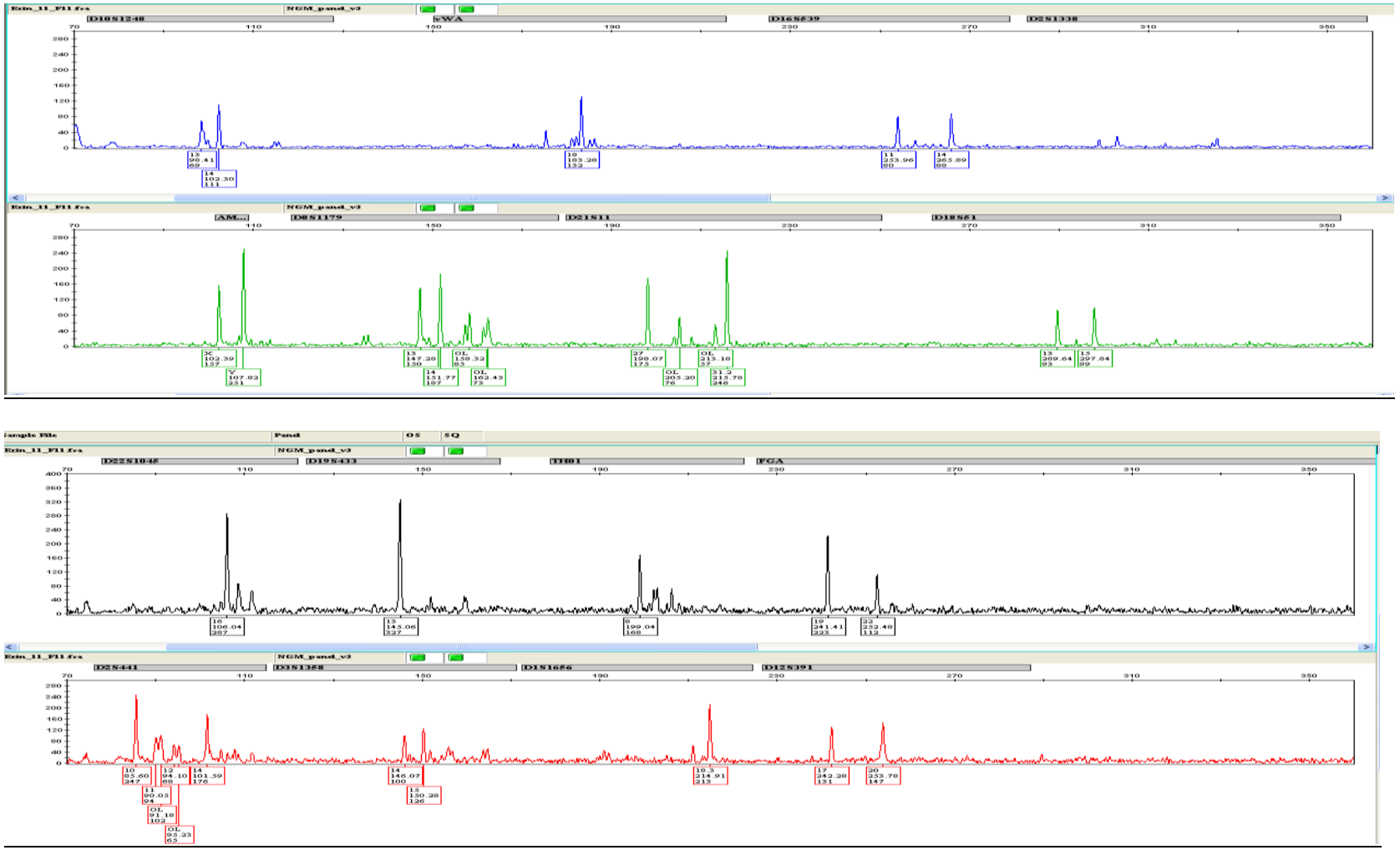

Figure 25: Electropherogram from towel with transfer from a T-shirt with 5 ml of blood during machine laundering.

Figure 26: Electropherogram from towel with transfer from a T-shirt with 10 ml of blood during machine laundering

Figure 27: Electropherogram from female underwear with transfer from being laundered in a secondary load after a primary load with a bloody shirt.

Figure 28: Electropherogram from a towel with transfer from being laundered as a tertiary load after a primary load with a bloody shirt