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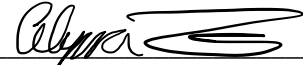


University of New Haven
Honors Program

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Investigating the Efficacy of DNA Damage with Bleach in
Forensic Laboratories and at Crime Scenes

Alyssa Tuccinardi

A thesis presented in partial fulfillment of the requirements of the Undergraduate Honors
Program at the University of New Haven

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Date

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ABSTRACT

Household/commercial bleach (6% NaOCl, sodium hypochlorite) degrades DNA through oxidative damage, production of chlorinated base products, and cleavage of DNA strands (breaking it into smaller and smaller fragments). The presence of these lesions significantly impacts the ability to generate a full genetic profile from an evidentiary sample. In fact, knowledge of the damaging effect of bleach on DNA is the basis for its use in forensic laboratories to clean workbenches and prevent cross-contamination of samples between cases. Additionally, bleach is used intentionally by criminals to clean up crime scenes and destroy DNA evidence. A previous study demonstrated that bleach has a decreased effect on native DNA that is still encompassed within a body fluid (compared to naked DNA that has already been extracted) (Ambers et al. 2014). This research project expanded on the previous study, with an increased sample size and expanded data set. Numerous variables were tested, including dried blood, wet (uncoagulated) blood, native DNA, naked DNA, dried semen, wet (liquid) semen, and varying concentrations of bleach. DNA in whole human blood or semen (native conformation) and extracted (naked) DNA were immersed in two different concentrations of bleach for a 1-hour exposure period. Solid-phase DNA extraction and human-DNA-specific quantification revealed that sufficient quantities of DNA were recovered for STR typing, for both native and naked DNA templates and after exposure to both bleach concentrations (with higher DNA recovery from native samples vs. naked templates).

CHAPTER I

Introduction

In forensic casework, there are three major factors which significantly impact successful recovery of a DNA profile from evidence, including low-quality (damaged/degraded) DNA, low quantity DNA (often referred to as low copy number, LCN), and the presence of endogenous or environmental inhibitors. The latter two factors have largely been mitigated by recent advances in instrumentation, “increased sensitivity” methods, and improvements in DNA extraction techniques. However, DNA damage/degradation is inherent in an evidentiary sample when it arrives in the laboratory. The degree and spectrum of DNA damage present in a sample depends on the environment to which it was exposed and the length of exposure time. Significant damage or alteration to the primary molecular structure of DNA is problematic because polymerases stall at damaged/altered sites, preventing amplification (and therefore analysis) of target loci.

The mechanisms of DNA damage are diverse and can be divided into four major categories: depurination, crosslinking, base alteration, and strand breakage. In the natural environment, ultraviolet light, acidity, heat, and humidity all contribute to various forms of damage in the molecular structure of DNA (Ambers et al. 2014). In addition to environmental insult, chemicals can be used to damage DNA. In fact, bleach is used intentionally by criminals to clean up crime scenes and destroy DNA evidence. Furthermore, knowledge of the damaging effect of bleach on DNA is the basis for its use in forensic laboratories to clean workbenches and prevent cross-contamination of samples between cases.

Bleach (sodium hypochlorite, NaOCl) degrades DNA through oxidative damage and production of chlorinated base products. Exposure to increasingly higher concentrations of NaOCl eventually causes cleavage of DNA strands, breaking it into smaller and smaller fragments. Although decontamination procedures in a forensic laboratory setting are carried out with diluted bleach, criminals are likely to use much higher concentrations in an effort to destroy DNA evidence. Interestingly, recent studies indicate that the degradative effects of bleach on DNA (as well as the rate of damage) varies quite substantially depending on the physical state of a body fluid (Ambers et al. 2014; Kemp and Smith 2005). More importantly, preliminary results suggested that bleach has a decreased effect on 1) dry coagulated blood (compared to wet, uncoagulated blood), and 2) native DNA that is still encompassed within a body fluid (compared to naked DNA that has already been extracted from a stain or body fluid). Further exploration is needed to understand how the concentration of bleach used and exposure time affects DNA within various types of body fluids that are collected as evidence in criminal cases. The previous study's findings have value because they indicate that current decontamination methods using bleach in the laboratory may not be as effective as believed (at least for DNA complexed with other materials). Further studies are warranted to determine if native DNA contamination in a laboratory is neutralized effectively with bleach. Additionally, it is often assumed that if a criminal has cleaned a crime scene with bleach, any underlying DNA evidence has been destroyed (which might prevent crime scene technicians from swabbing the area and submitting samples to laboratories for DNA analysis).

Literature Review

Contamination prevention measures in forensic DNA laboratories

Vandewoestyne (2011) evaluated the precautions needed to minimize the risk of contamination in forensic DNA casework. This study examined the benchtop surfaces, air, tools, and equipment being used in forensic DNA typing laboratories to determine if they are possible sources of contamination. Results from sampling of air concluded that air is not likely a contributing variable to DNA contamination, due to the absence of detection of alleles in the tested samples. The tools, surfaces, and equipment studied did reveal evidence of contaminant human DNA, as at least one foreign (exogenous) allele was recovered during DNA typing. Some tested samples contained more alleles than others, demonstrating that some objects or surfaces are more prone to contributing to intralaboratory contamination. After initially detecting the presence of DNA contamination, Vandewoestyne's team then performed a decontamination process on the tools, surfaces, and equipment to determine if these processes effectively removed or destroyed the contaminating DNA. Post-decontamination testing revealed that the level of contamination present on various objects and surfaces was no different than before the decontamination process, indicating that some cleaning/decontamination approaches are not successful.

Appropriate cleaning of examination areas and equipment prior to forensic DNA analysis is crucial in order to 1) decrease the risk of unintended contamination of crime scene evidence, and 2) prevent cross-contamination of samples between criminal cases. One study (Gall 2015) identified that cleaning, sterilization, and DNA decontamination are three completely different processes and do not function as a single (combined) process.

Each individual process has applicable steps and regulations that are required to be followed thoroughly and consistently in order to avoid unintended cross-contamination. This study also determined that, in a clinical environment, DNA cannot be completely eliminated, although its presence can be minimized through these processes.

Body fluids (e.g., blood, urine, semen) may contain blood-borne pathogens (e.g., HIV, hepatitis), and individuals working in the healthcare field and forensic casework laboratories must be aware of these pathogens to prevent exposure and reduce the chances of infection. Both chemical and physical cleaning methods exist for decontaminating areas that have been exposed to body fluids that potentially contain pathogenic infectious agents. Chemicals such as isopropyl alcohol, ethyl alcohol, bleach, and iodophors are used to mitigate and/or remove such biological hazards from laboratory environments. Among the physical decontamination methods used include “cleaning surfaces” via exposure to ultraviolet (UV) light or to ionizing radiation.

Kampmann (2017) examined various ways to remove DNA from hard surfaces in a forensic laboratory. Hard surfaces were exposed to 10 ng, 1 ng, 500 pg, and 100 pg of DNA in separate areas. Each area was cleaned via one of the following methods: 1) with water only; 2) with water and 96% ethanol (EtOH); 3) with 96% ethanol (EtOH); 4) with 10% Klorrent Disinfectant (Novadan®); 5) with 3% Klorrent Disinfectant; or 6) no cleaning at all. Results demonstrated that DNA was still detectable on the hard surfaces of laboratory workbenches, even after cleaning (and regardless of the cleaning method utilized). The various cleaning chemicals used did damage some of the DNA, but did not completely destroy it.

Forensically relevant body fluids – Blood and semen

Creamer (2005) cleaned wet and dried bloodstains on tiles with either bleach (sodium hypochlorite, NaOCl) or water. The luminol test (a presumptive test for blood) was then performed on these tiles over a period of time after the tiles were cleaned. Results showed that tiles that had been cleaned with bleach displayed an immediate decrease in chemiluminescence followed by an increase in chemiluminescence. Eight (8) hours after cleaning the tiles with bleach, the results became statistically insignificant.

Castello (2009) discussed the impact that bleached bloodstains have on luminol tests and the effect of bleach on various types of surfaces that blood may be deposited on. Luminol was chosen for this study because it is commonly used by forensic scientists to detect traces of blood. Furthermore, luminol has a high sensitivity for oxidizing agents, and bleach is an oxidizing agent. Castello and his team evaluated chemiluminescence of bloodstains treated with bleach and luminol compared to luminol only. Results showed that the type of surface affects both the drying period and the luminol reaction when bleach is present.

Bittencourt (2009) examined the ability to obtain an STR (DNA) profile from blood samples that are present in trace amounts (small quantities), which is typical at many crime scenes, especially if the perpetrator has attempted to clean up the blood prior to its discovery. The purpose of their examination was to determine if DNA could still be recovered from blood samples that have been subjected to washing or cleaning. Presumptive tests were performed to verify that the sample being examined was blood, and then the bloodstains were subjected to cleaning with and without bleach containing chlorine. Blood samples were deposited on a variety of fabrics commonly used in clothing

or bedding, in order to determine if the type of fabric interferes with or has any effect on DNA recovery and generation of an STR profile. DNA was extracted from the samples and amplified. Results indicated that small amounts of DNA could be recovered from large sections of each fabric, and smaller patches within the fabrics maintained even larger amounts of DNA, even after treatment and exposure to the cleaning conditions.

When examining sperm, there is a difference between sperm cells that are ejaculated in semen and testicular spermatozoa. Previous research has shown that there is more DNA damage present in ejaculated spermatozoa than testicular spermatozoa (Jesitus 2011). Jesitus et al. (2011) examined the difference between DNA damage in sperm types and concluded that high levels of DNA damage tend to be present in ejaculated spermatozoa, whereas a lower degree of DNA damage is displayed in testicular spermatozoa. This could have important implications for sexual assault cases, in which ejaculated semen containing sperm cells may be collected as evidence.

According to McDonald (2015), traces of sexual assault evidence are typically found on or in a victim's body, on clothing or bedding, and at the location where the assault occurred. In this study, the researchers examined how semen is generally detected and the current methods forensic scientists use to preserve and collect the evidence. A widely used presumptive test for semen is the acid phosphatase (AP) test, which detects the enzymatic activity of the protein acid phosphatase, which is present in high levels in semen samples. Confirmatory testing may involve histological staining techniques, e.g., the Christmas Tree Stain, which utilizes red and green dyes to differentially stain the head and tail of sperm cells to identify them in the presence of epithelial (skin) cells. Another test, the Rapid Stain Identification Test, assays a sample for the presence of semenogelin (a major component

of seminal fluid). Since many victims wash their clothes and bodies after a sexual assault or rape has occurred, this obviously damages any evidence present (including DNA contained within the sperm heads). In order to generate a DNA profile, victims must refrain from washing their clothes and bodies after the incident. However, results from McDonald's research show that semen can be detected on clothing even after washing, an important consideration in criminal casework.

Human semen is composed of four different components, including: 1) fluids produced in the prostate gland, 2) seminal fluid from the vesicles, 3) fluid from the testicles and the epididymis, and 4) fluid from the bulbourethral and urethral glands. The majority of fluid is produced in the seminal vesicle (Mandal 2019). Males normally ejaculate around 2-3 mL of fluid and only 10% of that is semen. Oxidative stress that occurs such as smoking, radiation, and drinking alcohol can cause DNA damage in semen cells, leaving sperm to be dysfunctional or can lead to sperm death. Reactive oxygen species (ROS) naturally present in the body's cells alter and damage DNA in sperm. According to Agarwal (2016) high oxidative stress in men has been associated with DNA fragmentation.

Generating damage in deoxyribonucleic acid (DNA)

Ambers et al. (2014) developed protocols to damage DNA in its native state, created a pool of individual samples for DNA repair, and assessed the ability of the PreCR Repair Mix (New England BioLabs) to repair the damaged DNA. This study explored multiple different degradation methods, such as: 1) oxidative damage via Fenton reaction or treatment with potassium permanganate, 2) depurination via exposure to high heat and humidity, 3) environmental exposure, 4) oxidative damage via peroxide, and 5) oxidative

damage via bleach. With the bleach protocol, the DNA was damaged due to the cleaving of phosphodiester bonds and fragmenting of the DNA into smaller pieces; however, full STR profiles were able to be recovered even after bleach treatment. These results were reported for DNA in both native and naked forms (with human bloodstains), and indicated that although damage did occur to the DNA molecule, some DNA fragments remained intact and therefore could still be recovered/profiled. This research explains several ways in which DNA can be damaged, and results posed many other questions regarding the decontamination processes used in forensic laboratories and the need to identify more effective ways to remove DNA contamination from laboratory spaces.

Kemp (2004) examined the presence of contamination on the surfaces of bones and teeth which, when detected, can result in an erroneous identification or a false exclusion. The presence of contaminant (exogenous) DNA interferes with ancient (endogenous) DNA because the ancient DNA is highly degraded, exists in low quantities, and is therefore more difficult to detect than higher quality or higher quantity contaminant DNA. The goal of Kemp's study was to eliminate or minimize the amount of surface contamination present on skeletal remains prior to DNA extraction. Sodium hypochlorite (bleach) was used to "destroy" or wash away the contaminating DNA present on the skeletal remains. Results demonstrated that if bones or teeth are submerged in 6.0% sodium hypochlorite for 15 minutes prior to DNA extraction, exogenous surface contamination is eliminated.

Szkuta (2015) studied the effect of sodium hypochlorite (bleach) remaining on surfaces after treatment with bleach of varying concentrations. This study observed the degradation effect of sodium hypochlorite on the quantity and quality of DNA recovered from those surfaces both pre-exposure and post-exposure. From this research, it was

determined that there was no effect on DNA quality or quantity on surfaces not exposed to bleach; however, samples which did come in contact with bleach were slightly degraded. For decontamination, Szkuta recommended that laboratories use high concentrations of sodium hypochlorite to remove or destroy surface DNA contamination.

Noel (2019) examined repeatedly washed semen stains on bedding and clothing in sexual assault cases. Different washing methods were explored, including different types of washing machines, different laundry detergents, and the length (time frame) of the wash cycle. This has important applications to sexual assault cases because if the bedding and/or clothing of the victim is washed or bleached, this decreases the quantity of DNA present and may also damage/degrade the DNA, complicating a forensic scientist's ability to recover the perpetrator's DNA profile. With the bedding samples, the bleach hindered the detection of semen with the PSA test. However, after performing DNA extraction and quantification, a large amount of DNA was still able to be recovered. Washing conditions for this sample included immersion in an 8% bleach solution. Results from this research were congruent with previous research studies which indicate that body fluid stains that have been previously washed could still yield sufficient DNA to generate a genetic profile of the assailant.

Forensic implications

Stevens (2008) addresses the controversy between real-life forensic science and what is depicted in popular television shows. He noted that criminals are learning how to cover up their crimes and destroy evidence from these television shows. He provides an example in which a girl was murdered, and the suspect used bleach to clean up all of her

blood. He quoted a criminalist stating, “Today the use of bleach, which destroys DNA, is not unusual in a planned homicide.”

Crime scene decontamination

After investigators and forensic scientists are finished collecting evidence from a crime scene, it must be thoroughly cleaned and sterilized before the room, building, or site can be reopened for human contact. Crime scenes are generally cleaned with bleach and other recommended cleaning supplies, according to standards developed by the Occupational Safety and Health Administration (OSHA). Crime scenes are considered to be a biohazard because of blood-borne pathogens that may exist within body fluids spilled at the scene. However, in addition to cleaning of crime scenes by investigative authorities, criminals may also attempt to clean up the scene in order to destroy or eliminate incriminating evidence.

PCR amplification of DNA

Champlot (2010) examined problems related to DNA concentration and fragment sizes when using PCR for typing of biological samples. Additional complications arise when PCR co-amplifies contamination in the sample, from laboratory surfaces, carry-over contamination from previous samples, and contamination in PCR reagents (Champlot 2010). The study determined that current decontamination methods are not effective at degrading DNA, cannot be applied to reagents, or interfere with PCR. The most effective decontamination was achieved when multiple treatments were used. Data from this

research suggests that there is no single decontamination method useful for application to all possible contamination sources that may be encountered in PCR.

PCR is a highly effective method for making copies of low quantities of DNA, sometimes even when the template DNA is damaged. Generally, PCR amplification of target loci in forensic casework requires an optimum amount of input template DNA (1 ng) and 30 amplification cycles (Alaeddini 2010). In degraded samples, smaller (shorter) DNA fragments are more likely to be intact (and amplifiable) than longer fragments. For degraded samples, new “mini” primers are more successful because they can be positioned closer to the target locus, reducing the amplicon size required for completion of the copying mechanism.

Andrews (1994) researched the feasibility of PCR amplification of DNA from washed stains. In this study, items of clothing that contained blood, semen, and saliva stains were examined and subjected to a variety of washing methods. Post-washing, DNA extraction and genetic typing was attempted. Results demonstrated that the washed samples still contained a measurable amount of DNA, although detecting the presence and location of each stain was a challenge after washing. For all samples in the study, over 1 ng of DNA per microliter (μL) was recovered, which is more than enough DNA to generate a full genetic profile using current (modern) technologies.

Tobe (2017) studied the use of direct PCR on semen and spermatozoa, as well as the development of a differential isolation protocol that is generally used in cases of alleged sexual assaults. This research also investigated a new method of amplifying DNA in semen samples without first carrying out DNA extraction procedures (a new approach for implementation into the forensic science community). Proponents of this new method

report that it will reduce the amount of time required to process DNA samples and can increase the sensitivity of detection during PCR. However, preliminary results show that direct PCR approaches amplify all DNA in an evidentiary sample, not just DNA from sperm cells, and hence can result in complex DNA mixtures (which are difficult to interpret). Additional sources of DNA in a sexual assault evidentiary sample are epithelial cells from the victim's vagina, epithelial cells in semen, and white blood cells (leukocytes) in semen.

Sexual assault evidence

The article "DNA Evidence in Rape Cases and The Debbie Smith Act" examines a legal directive that was put into place in 2004 after a woman was sexually assaulted by a man who robbed her house and then raped her (Telsavaara and Arrigo, 2006). The purpose of enacting this law was to address issues of analyzing DNA evidence from rape kits that had been sitting untested in forensic laboratories for extended periods of time across the country. This article specifically highlighted the time it took to generate genetic profiles from collected rape kits. Telsavaara states that there was a lack of properly-trained individuals and available funding to facilitate examination of all of the rape kits collected. The Debbie Smith Act also permitted the CODIS database to be updated with profiles generated from rape kits so that perpetrators can be identified and to potentially exonerate those who have been wrongfully convicted of sexual assault crimes in the past. The processing of rape kit evidence has recently increased in scope and productivity, slowly reducing the rape kit backlog that currently exists. The increased sensitivity of many

instruments and improvements in technology allows forensic scientists to recover DNA profiles from dilute, low quantity, and/or degraded DNA.

Research objectives

The goal of this research was to investigate differences in the efficacy of bleach in generating damage to native and naked DNA templates. Specifically, experiments were designed to determine if bleach causes more damage to DNA when the DNA has already been extracted from a body fluid (i.e., in its naked conformation) or when it is still encompassed within a body fluid (i.e., in its native conformation). Blood and semen were selected because these are the two most common sample types recovered from crime scenes.

Ultimately, investigation into this research topic is of particular interest because 1) bleach is considered the “gold standard” for cleaning and sterilizing laboratory workbenches between analysis of different items of evidence, as well as between cases (to prevent cross-contamination), and 2) bleach is often used by perpetrators to clean up crime scenes and destroy DNA evidence. This study will also investigate differences in the efficacy of household (commercial) bleach and laboratory grade bleach in damaging and destroying DNA in body fluids and on laboratory surfaces.

CHAPTER II

Materials and Methods

Blood and semen were selected for this research because they are the most common biological body fluids encountered at crime scenes (especially in association with violent offenses). Also, criminals bleach crime scenes in an attempt to remove visible bloodstains from floors and walls, as well as from clothing, carpet, or bedding. Whole human blood was collected from a volunteer, in accordance with UNH Institutional Review Board (IRB) guidelines and approved IRB Protocol # 2019-048 (**Appendix I**). Human semen was collected from volunteers, under the approved IRB Protocol # 2019-101 (**Appendix I**). Informed consent was obtained for all samples collected, and a copy of the IRB-approved consent form is included in **Appendix II**.

Both extracted (naked) DNA and native DNA (still contained within blood or semen) were immersed in two different concentrations of bleach: 1) 10% household/commercial bleach (0.6% NaOCl), which is consistent with the concentration used by forensic casework laboratories to decontaminate workbenches; and 2) 100% household bleach (6% NaOCl), which is more likely to be used by criminals in an effort to clean up bloodstains and destroy DNA evidence. For all experiments, the ratio of bleach-to-blood or bleach-to-semen was standardized (10 times the volume of bleach to the volume of blood/semen was used). An overview of the protocols used to generate DNA damage in blood samples are outlined in **Figure 1**. The same approach was used for semen samples. Control DNA samples from blood and semen (i.e., samples not exposed to bleach) were also processed for comparison.

DNA extractions were performed using the QIAamp® DNA Investigator Kit (Qiagen Inc, Germantown, MD) and a 25µl elution volume. A sample identification key (which explains the coding, letters, numbers, and acronyms used) is included in **Appendix III**. The codes and acronyms used in this key signify the physical state of the DNA (naked vs. native), the body fluid used (blood or semen), the physical state of the body fluid (wet vs. dry), and the concentration of bleach used (10% vs. 100%).

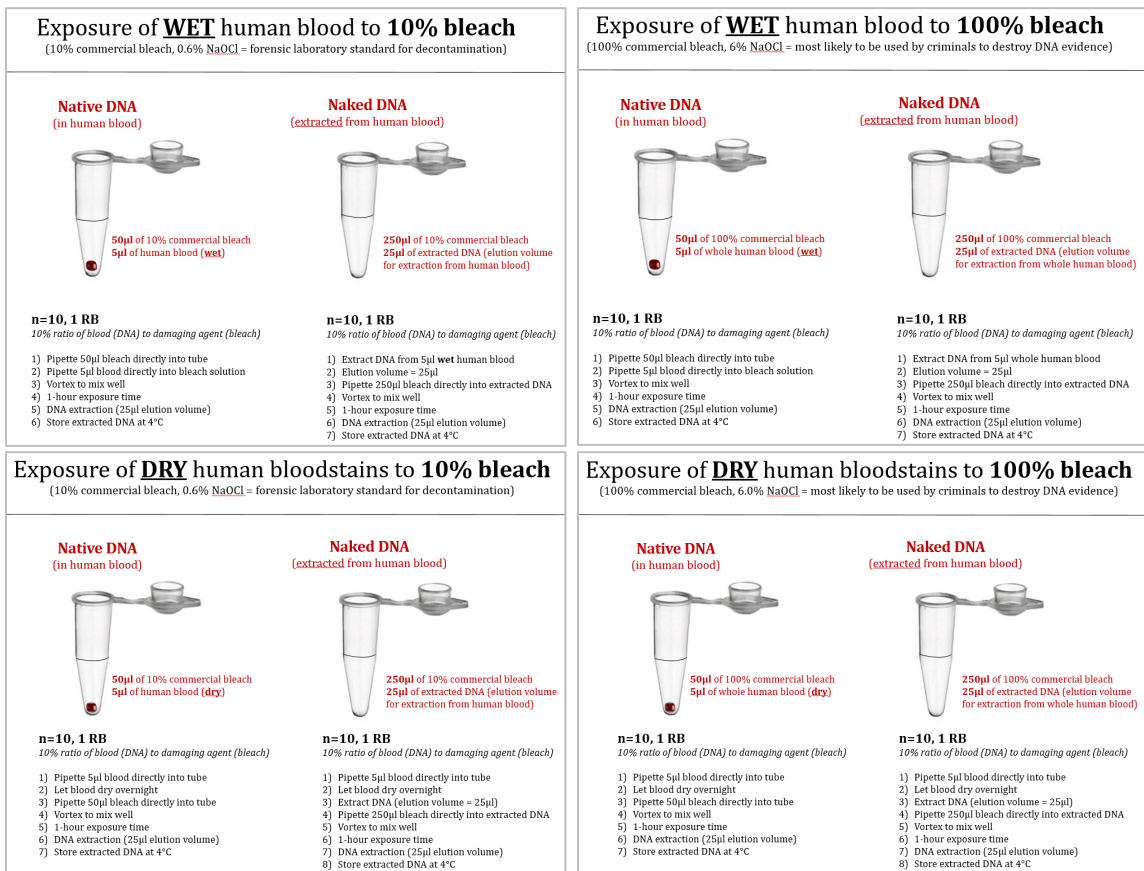


Figure 1. Protocols for bleach damage of native DNA (still contained within human blood) and naked DNA (which has already been extracted). A 10:1 ratio of bleach: blood was used for all experiments. The same protocols were used for semen samples.

Extracted DNA was quantified using the Quantifiler™ Human DNA quantification kit (Applied Biosystems, Thermo Fisher Scientific). This quantification method is based

on the polymerase chain reaction (PCR), a reaction that is inhibited and/or stalled by the presence of DNA damage. A standard curve was generated via a dilution series of a known DNA standard (200 ng/ μ l) included with the quantification kit (**Appendix IV**). Post-quantification, the standard curve was checked for performance quality (including evaluation of the R^2 value, slope, and y-intercept) to ensure the values fell within the acceptable range (**Appendix IV**). The non-template control (NTC) and extraction reagent blanks (RBs) were checked for absence of contamination. For all DNA samples, the internal positive control (IPC) was verified for each well on the reaction plate to ensure that amplification was successful and operating at optimal efficiency.

CHAPTER III

Results and Discussion

After treatment with the damaging agent (10% or 100% household bleach), results show that DNA damage occurs more in naked DNA samples than in native DNA samples. As shown in **Figure 2**, the average total DNA recovery after treatment with 10% household bleach (0.6% NaOCl) was 3.16 ng for naked samples and 106.88 ng for native templates, demonstrating a strong correlation between the two variables (naked vs. native) and the degree of damage that occurred. DNA recovery after treatment with 100% household bleach (6% NaOCl) was 2.17 ng for naked samples and 115.49 ng for native templates, again indicating a strong correlation between the physical state of DNA and the damage observed (**Figure 3**). T-test results were significant for both data sets ($p < 0.05$).

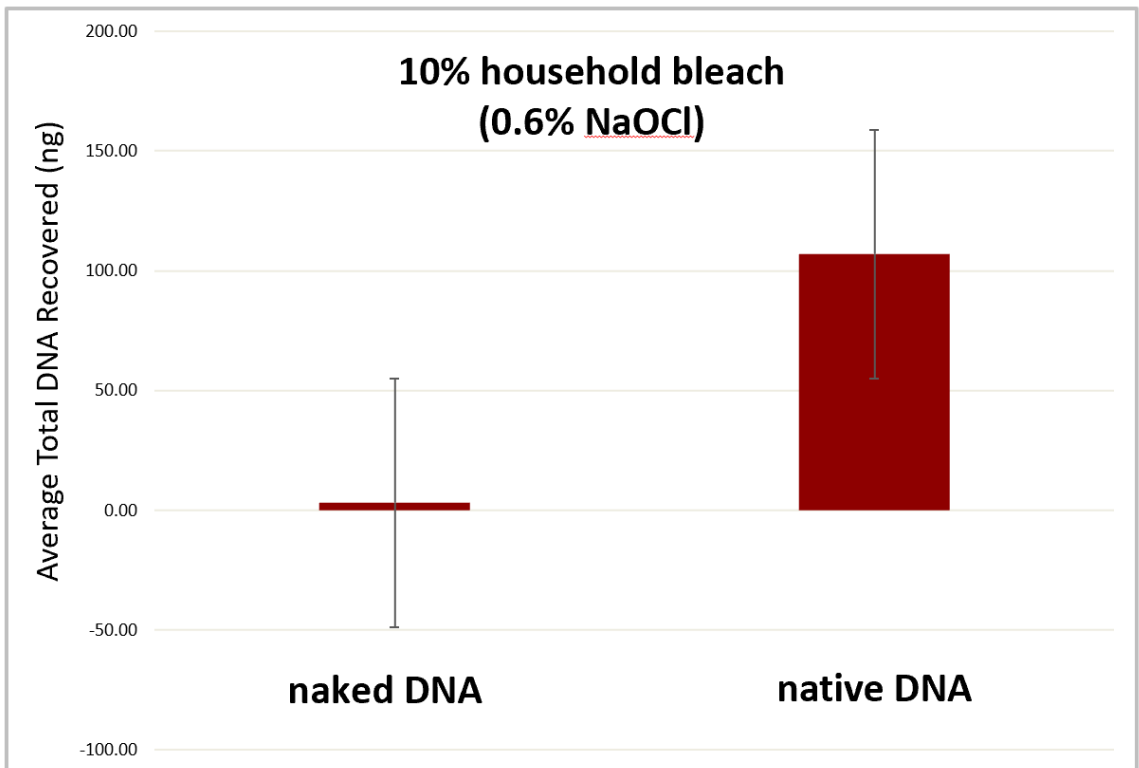


Figure 2. Average DNA recovery (ng) from naked samples (n=20) and native samples (n=20) treated with a 10% dilution of household bleach (0.6% NaOCl) for a 1-hour exposure period (total n=40).

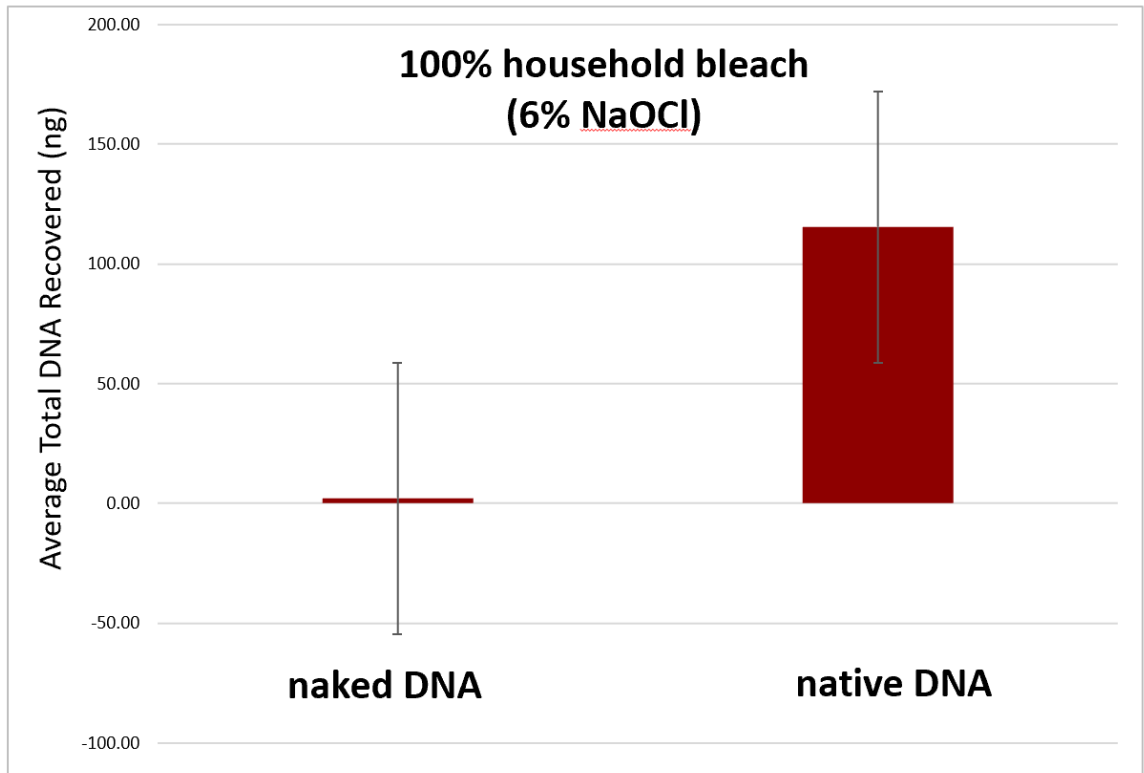


Figure 3. Average DNA recovery (ng) from naked samples (n=20) and native samples (n=20) treated with 100% household bleach (6% NaOCl) for a 1-hour exposure period (total n=40).

Differences in the effects of bleach on DNA in blood could be explained by understanding the physical packaging of DNA, as it exists within human cells or body fluids. In living organisms, nuclear DNA is not a “naked” molecule. In its native conformation, DNA is a supercoiled structure that is highly packaged into chromatin and is associated with a variety of other molecules. Hence, the manner or degree in which damage occurs to DNA in its native, complexed form is likely quite different than in its naked counterpart. Native DNA may be afforded some protection from damage because it is surrounded by a cellular milieu of proteins, lipids, carbohydrates, and other nucleic acids (RNA).

In addition to the physical state of DNA (naked vs. native), another factor explored as a potential variable related to the degree of DNA damage that can be caused by bleach was the physical state of the blood (dried vs. wet) upon treatment. **Figure 4** depicts average DNA recovery from 5 μ l whole human blood after being treated with 10% household bleach for 1-hour; total average DNA recovery was 64.29 ng in the dried state and 45.76 ng in the wet state. After treatment with 100% household bleach for 1-hour, total average DNA recovered from 5 μ l dried and wet (uncoagulated) blood samples was 48.72 ng and 68.95 ng, respectively (**Figure 5**). Differences in DNA recovery for both treatment conditions were not significant ($p > 0.05$), indicating that the physical state of blood does not affect the amount of DNA damage that can be caused by bleach.

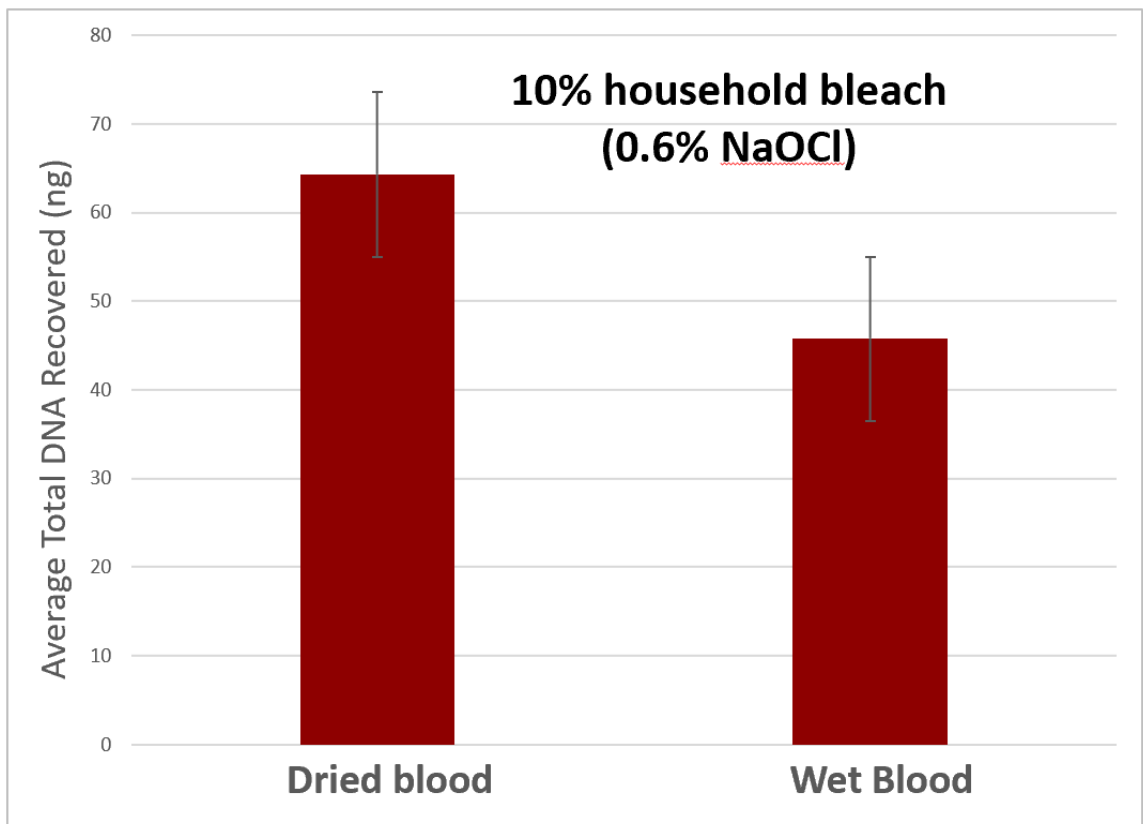


Figure 4. Average DNA recovery (ng) from dried blood vs. wet (uncoagulated) blood (n=40) after treatment w/10% household bleach (0.6% NaOCl).

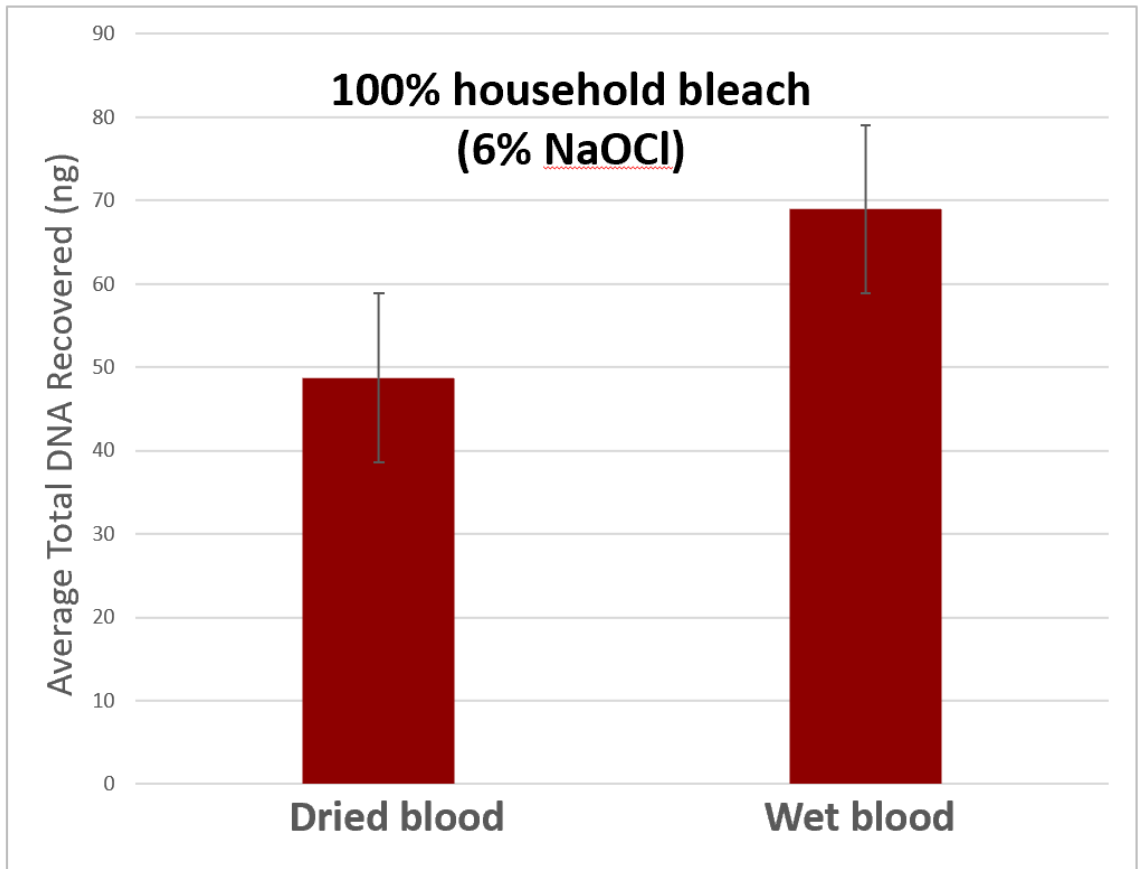


Figure 5. Average DNA recovery (ng) from dried blood vs. wet (uncoagulated) blood (n=40) after treatment w/100% household bleach (6% NaOCl).

In addition to human blood, fifty-five (55) semen samples were processed using the same damaging protocols applied to blood samples (i.e., exposure of both native and naked DNA from semen to 10% bleach and 100% bleach, in both wet and dry states). The samples are stored in the freezer in the DNA laboratory in Dodd’s Hall at the University of New Haven, but DNA quantification was unable to be performed due to the closure of campus in response to the corona virus (COVID-19) pandemic. DNA quantification results for all control samples used in this study are reported in **Appendix V**.

CHAPTER IV

Conclusions

The goal of this research was to investigate differences in the efficacy of bleach in generating damage to native and naked DNA templates. Results indicate that current decontamination methods using bleach in the laboratory may not be as effective as perceived (at least for DNA complexed with other materials). Additionally, it is often assumed that if a criminal has cleaned a crime scene with bleach, any underlying DNA evidence has been destroyed (which might prevent crime scene technicians from swabbing the area and submitting samples to laboratories for DNA analysis). Hence, this research will impact the forensic science community by demonstrating that amplifiable DNA often can still be recovered from human blood that has been exposed to bleach, especially if the DNA is still encompassed in its native tissue upon initial exposure (i.e., still protected within the body fluid). Decontamination of laboratory workbenches may actually be partially due to physical removal of DNA from a surface (“wiping away”) as opposed to chemical destruction or damage.

Future studies

Future studies will focus on: 1) assessing bleach’s damaging effects on DNA in semen (another common body fluid recovered from crime scenes, and with specific application to sexual assault cases); 2) investigation of the physical removal (wiping) variable; and 3) comparison of the efficacy of household-grade (commercial) bleach and laboratory-grade NaOCl in causing chemical damage to DNA. In addition, longer exposure periods to bleach could be explored to see if time impacts the degree of DNA damage.

Advanced knowledge of the use of household bleach and its effects on DNA in bodily fluids can assist law enforcement and forensic scientists in assessing which evidentiary items may provide the best probability of typing success. This can ultimately lead to more cases being solved. Lastly, other types of cleaning solutions (besides bleach) could be investigated to see if there is a more effective solution for cleaning and decontaminating forensic laboratories between cases.

APPENDIX I



University of New Haven

IRB Disposition Form

Date: Nov 21, 2019

To: Dr. Ambers

From: Dr. Alexandria Guzmán, IRB Chair

AEG

Proposal Title: Investigation of the Efficacy of DNA Damage with Bleach in Forensic Laboratories and Sexual Assault Cases

Protocol Number: 2019-101

Review by: Committee _____ Date of Meeting _____
Expedited Procedure ___AEG___

The IRB has approved the proposed use of human participants in this project.

__XX__ The proposal is approved as revised.

___ The proposal is approved with the following minor stipulations. If you agree to the condition(s) of approval, please sign one copy and return it to the IRB chair. If the condition(s) of approval are not clear or are unacceptable to you, please contact the IRB chair.

___ The proposal has not been approved in its current form. The committee advises that the revisions below be made and the application be resubmitted for further IRB review. If the suggestions for resubmission are not clear or are unacceptable to you, please contact the IRB chair.

___ The proposal has not been approved. Reasons for this decision are provided below.

Project Expires on

NOV - 1 2020

University of New Haven Institutional Review Board

PLEASE NOTE

1. **Approval of the project will expire on 11/1/20**. Federal regulations (DHHS) and UNH policy require that you submit a “Continuing Review Form” at that time if continued approval is desired. A copy of the “Continuing Review Form” is attached.
2. If the research procedures are altered from the description in the proposal reviewed, you must submit a “Request for Revision Form” to the IRB.
3. Upon approval of the study, a consent document will be stamped with an expiration date. Only this document may be used when enrolling subjects. Studies extending beyond the expiration date must be submitted for a continuation review. Any changes in the consent form must be approved by the IRB.
- 4 For projects with multiple data collection points, informed consent must be obtained at EACH data collection session.
5. When participant recruitment and data collection are completed for this project, federal regulation and UNH policy require that you submit a “Research Completion Form” to the IRB. After this form is submitted, if you wish to recruit more participants or collect more data for this project, you must submit a new IRB Application form for review.
5. Should any modifications be made in the approved project, a “Request for Revision” form must be submitted to the IRB.
6. If any adverse event or data breach occurs during participant recruitment or data collection an “Adverse Event/Data Breach” form must be submitted to the IRB.
7. If any problems arise concerning the welfare of subjects in the projects, please contact me (irb@newhaven.edu).
8. It is the responsibility of the Principal Investigator to ensure that any person that joins the research team after initial IRB approval be certified prior to interacting or intervening with human participants or their data.

Best wishes for the successful completion of your research.

Project Expires on

NOV - 1 2020

University of New Haven
Institutional Review Board



University of New Haven

IRB Disposition Form

Date: June 7, 2019

To: Dr. Angela Ambers

From: Dr. Alexandria Guzmán, IRB Chair

AEG

Proposal Title: Bleach Decontamination in the Forensic Laboratory and at the Crime Scene: Investigating the Efficacy of DNA Damage in Native versus Naked Templates

Protocol Number: 2019-048

Review by: Committee _____ Date of Meeting _____
Expedited Procedure ___AEG___

The IRB has approved the proposed use of human participants in this project.

The proposal is approved as revised.

The proposal is approved with the following minor stipulations. If you agree to the condition(s) of approval, please sign one copy and return it to the IRB chair. If the condition(s) of approval are not clear or are unacceptable to you, please contact the IRB chair.

The proposal has not been approved in its current form. The committee advises that the revisions below be made and the application be resubmitted for further IRB review. If the suggestions for resubmission are not clear or are unacceptable to you, please contact the IRB chair.

The proposal has not been approved. Reasons for this decision are provided below.

PLEASE NOTE

1. **Approval of the project will expire on 6/1/20**. Federal regulations (DHHS) and UNH policy require that you submit a “Continuing Review Form” at that time if continued approval is desired. A copy of the “Continuing Review Form” is attached.
2. If the research procedures are altered from the description in the proposal reviewed, you must submit a “Request for Revision Form” to the IRB.
3. Upon approval of the study, a consent document will be stamped with an expiration date. Only this document may be used when enrolling subjects. Studies extending beyond the expiration date must be submitted for a continuation review. Any changes in the consent form must be approved by the IRB.
4. For projects with multiple data collection points, informed consent must be obtained at EACH data collection session.
5. When participant recruitment and data collection are completed for this project, federal regulation and UNH policy require that you submit a “Research Completion Form” to the IRB. After this form is submitted, if you wish to recruit more participants or collect more data for this project, you must submit a new IRB Application form for review.
5. Should any modifications be made in the approved project, a “Request for Revision” form must be submitted to the IRB.
6. If any adverse event or data breach occurs during participant recruitment or data collection an “Adverse Event/Data Breach” form must be submitted to the IRB.
7. If any problems arise concerning the welfare of subjects in the projects, please contact me (irb@newhaven.edu).
8. It is the responsibility of the Principal Investigator to ensure that any person that joins the research team after initial IRB approval be certified prior to interacting or intervening with human participants or their data.

Best wishes for the successful completion of your research.

Project Expires on

JUN - 1 2020

University of New Haven
Institutional Review Board

APPENDIX II

Informed Consent Form Blood and Semen Samples

University of New Haven

Title of Project: Investigation of the Efficacy of Bleach Damage in Forensic Laboratories and Sexual Assault Cases

Principal Investigator(s): Dr. Angie Ambers

Participant's Name (printed): _____

You are being asked to participate in a study involving "Investigation of the Efficacy of Bleach Damage in Forensic Laboratories and Sexual Assault Cases," conducted by the Forensic Science Department at the University of New Haven. Please read this consent form carefully, and ask questions (if necessary) before signing and agreeing to take part in this research.

In forensic casework, there are three major factors which significantly impact successful DNA profiling of evidence, including degraded DNA, low quantity DNA, and the presence of environmental inhibitors. Bleach is the "gold standard" method used in forensic casework laboratories to sterilize work surfaces to prevent cross-contamination of samples between cases. Additionally, bleach is used intentionally by criminals to clean up crime scenes and destroy DNA evidence. Although decontamination procedures in a forensic laboratory setting are carried out with diluted bleach, criminals are likely to use much higher concentrations in an effort to destroy DNA evidence. Recent studies indicate that the degradative effects of bleach on DNA vary quite substantially depending on the physical state of a body fluid (dry vs. wet). The purpose of this study is to further explore and understand how the concentration of bleach used or the exposure time affects DNA within two types of body fluids (blood, semen) that often are collected as evidence in criminal cases.

Participating in this study is voluntary, and will involve collection of one blood sample (from the faculty mentor, Dr. Angie Ambers) as well as semen from male volunteers. Collection of the blood sample will be conducted by a trained professional in the UNH Health Clinic. The semen sample collection technique is completely non-invasive (e.g. does not involve the use of needles) and is a normal physiological response associated with human sexual arousal. A sterile collection tube will be provided so that the semen sample can be collected voluntarily in the privacy of the home setting, at the donor's convenience.

All collected samples will be anonymized and labeled with a numerical sample identification code. Specific identifying or individualizing information will not be collected and/or recorded. A small subset of samples in this study may be profiled for DNA; however, most samples will only be subjected to quantification testing (not profiling), in order to simply determine the degree of DNA degradation caused by the bleach treatment. Any DNA profiles obtained will only be associated with an anonymous sample number and will not be directly associated with any

Project Expires on

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University of New Haven
Institutional Review Board

personally identifying information. The samples for this study are being used solely for academic research and not for criminal investigation purposes.

If at any time you decide to withdraw your voluntary participation in this research, your sample(s) will be immediately removed from the sample set and will be permanently destroyed (along with any associated data). Withdrawing from the study will not affect your current or future relationship with the investigators, the Forensic Science Department, or the University of New Haven (West Haven, Connecticut). Upon completion of this study, all research samples will be destroyed.

No compensation (monetary or otherwise) will be provided to participants of this study.

The researchers conducting this study are Dr. Angie Ambers and undergraduate Honors Thesis student, Alyssa Tuccinardi. Please ask any questions that you may have now. However, if you have questions about this study in the future, you may contact Dr. Ambers at aambers@newhaven.edu, office (203) 479-4583.

You will be given a copy of this form to keep for your records. The original signed consent form will be kept on file by the researchers for a minimum of 3 years after conclusion of the study.

Statement of Consent: I have thoroughly read the above information. I fully understand the purpose of this study, and I have received answers to all questions asked. I am over the age of 18 and consent to provide samples for this study. By signing this consent form, I indicate that I voluntarily choose to participate in this research.

Participant Signature _____ Date _____

Participant Name (printed) _____

Researcher Signature _____ Date _____

Researcher Name (printed) _____

If you have any questions or concerns regarding this study or your rights as a research participant and would like to talk to someone other than the researcher(s), contact the Chair of the Institutional Review Board (IRB) at UNH: irb@newhaven.edu, phone (203) 479-4562.

Project Expires on

NOV - 1 2020

University of New Haven
Institutional Review Board

APPENDIX III

Sample identification key:

Identification key for letters, numbers, and acronyms used throughout the experiments to signify the state of DNA (naked vs. native), state of the body fluid (wet vs. dry), concentration of bleach used (10% vs. 100%), and body fluid (blood or semen).

Acronym	Meaning
RB	Reagent Blank
1C	Wet blood samples with no household bleach
1-SC	Wet semen samples with no household bleach
1-NT-W-100	Sample 1, Native DNA, Wet blood, 100% household bleach
1-NK-W-100	Sample 1, Naked DNA, Wet blood, 100% household bleach
1-NT-W-10	Sample 1, Native DNA, Wet blood, 10% household bleach
1-NK-W-10	Sample 1, Naked DNA, Wet blood, 10% household bleach
1-NT-D-100	Sample 1, Native DNA, Dried blood, 100% household bleach
1-NT-D-10	Sample 1, Native DNA, Dried blood, 10% household bleach
1-NK-D-100	Sample 1, Naked DNA, Dried blood, 100% household bleach
1-NK-D-10	Sample 1, Naked DNA, Dried blood, 10% household bleach
1-S-NT-W-100	Sample 1, Semen, Native DNA, Wet semen, 100% household bleach
1-S-NK-W-100	Sample 1, Semen, Naked DNA, Wet semen, 100% household bleach
1-S-NT-W-10	Sample 1, Semen, Native DNA, Wet sample, 10% household bleach
1-S-NK-W-10	Sample 1, Semen, Naked DNA, Wet sample, 10% household bleach

APPENDIX IV

Standard dilution series for quantitative PCR (qPCR) reactions:

Standard	Concentration (ng/ μ L)	TE ⁻⁴ buffer (μ L)	DNA Amount	Dilution Factor
Std. 1	50	30	10 μ L stock	4X
Std. 2	16.7	20	10 μ L Std. 1	3X
Std. 3	5.56	20	10 μ L Std. 2	3X
Std. 4	1.85	20	10 μ L Std. 3	3X
Std. 5	0.62	20	10 μ L Std. 4	3X
Std. 6	0.21	20	10 μ L Std. 5	3X
Std. 6	0.068	20	10 μ L Std. 6	3X
Std. 8	0.023	20	10 μ L Std. 7	3X
NTC	-	20	-	-

NTC = non-template control

Quantifiler™ Human DNA quantification kit --- “Master Mix” calculations:

Reagents	Volume per sample (μ L)
Quantifiler™ PCR Reaction Mix	12.5
Quantifiler™ Human Primer Mix	10.5

Recommended ‘Standard Curve’ values for analyzing qPCR results:

Standard Curve Value	Expected Values
R ²	≥ 0.99
Slope	-3.3 to -2.9
Y-intercept	28.5

APPENDIX V

DNA quantification results for control (blood) samples:

Sample Name	Detector	Task	CT	Quantity (ng/μl)
1C	IPC	Unknown	27.59	
	Human	Unknown	26.3	4.22
2C	IPC	Unknown	27.52	
	Human	Unknown	26.6	3.39
3C	IPC	Unknown	27.42	
	Human	Unknown	27.03	2.47
4C	IPC	Unknown	27.39	
	Human	Unknown	27.26	2.07
5C	IPC	Unknown	27.4	
	Human	Unknown	27.83	1.37
6C	IPC	Unknown	27.24	
	Human	Unknown	27.34	1.96
7C	Human	Unknown	27.19	2.54
	IPC	Unknown	27.73	
8C	Human	Unknown	27.93	1.51
	IPC	Unknown	27.58	
9C	Human	Unknown	27.61	1.9
	IPC	Unknown	27.54	
10C	Human	Unknown	27.87	1.58
	IPC	Unknown	28.45	
11-RB-C	Human	Unknown	Undetermined	
	IPC	Unknown	28.42	

Sample	DNA Concentration (ng/μL)	Total DNA Recovered (ng)
1	4.22	105.50
2	3.39	84.75
3	2.47	61.75
4	2.07	51.75
5	1.37	34.25
6	1.96	49.00
7	2.54	63.50
8	1.51	37.75
9	1.90	47.50
10	1.58	39.50

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