Investigating the Correlation Between Textile Materials and Environmental Conditions on DNA Degradation in Bloodstains

1. Abstract

DNA is a crucial component of forensic science analysis, and its degradation can impact forensic samples by affecting the accuracy and reliability of results. This study investigates how different textile materials and environmental conditions influence DNA degradation and the efficiency of DNA extraction from blood stains. By understanding the limitations of DNA, forensic scientists can understand the limitations of their own analysis. 50 µl of swine blood will be placed on different textiles (faux leather, wool, polyester, and cotton) and left in varying environmental conditions for a set number of hours. These conditions are meant to represent real-world forensic scenarios. DNA extraction and quantification will be performed at designated time intervals to assess degradation rates and the effectiveness of extraction. Each sample will be prepared by cutting a 1 cm^2 square of stained fabric and placing the fabric in a microcentrifuge tube. Extraction of DNA will be conducted following a phenol-chloroform protocol. The analysis of DNA quality will be measured using Agarose Gel Electrophoresis and Nanodrop. Different textile materials and environmental conditions will significantly affect the rate of DNA degradation in bloodstains with natural fibers exhibiting higher degradation rates under harsher environmental conditions compared to synthetic fibers.

2. Introduction

Forensic investigations rely on the accurate analysis of biological evidence, however; this type of evidence is often unpredictable. DNA is one of the most unpredictable factors with outside variables like environmental conditions and material composition having an influence on its rate of degradation. Despite advances in forensic techniques, limited research explores the intersection of textile fabrics and environmental conditions. This research aims to determine whether different environmental conditions have varying effects on DNA degradation depending on the type of textile the bloodstain is on. By understanding the intersection of environmental factors and textiles, forensic scientists can enhance the accuracy of DNA-based identifications and increase the reliability of the results.

3. Background

DNA is one of the most powerful tools in forensic science, capable of unlocking identities, solving crimes, and connecting individuals across time and place. Deoxyribose nucleic acid is the building block of life holding the genetic material that makes people unique. In forensic science the study of DNA is critical to investigations. Forensic scientists choose to analyze DNA due to its stability, important genetic information, extensive storage database, and its extreme abundance. DNA is the most stable nucleic acid making it a reliable target for forensic analysis. The molecule's stability allows it to survive in harsh enviromental conditions which many forensic samples are exposed to.

Nuclear DNA is the genetic material found within the nucleus of a cell. It carries most genetic information that is important for forensic investigations and suspect identification. While important, it's prone to degradation due to its larger size and vulnerability to enzymatic activity. The level of degradation is determined by environmental conditions, sample age, and storage methods and consequences of nDNA degradation include reduced DNA recovery, allele dropout,

and increased risk of DNA mixtures. Degradation of DNA can be caused by enzymatic and non-enzymatic mechanisms. Enzymatic degradation involves nucleases which cleave the bond in DNA. Non-enzymatic degradation results from outside factors such as oxidative damage, chemical exposure, and physical stress. Physical stress includes temperature changes and humidity. High temperatures (>90°C) accelerate degradation, while cold temperatures slow down degradation. Humidity promotes hydrolysis, breaking down the hydrogen bonds with the DNA molecules. Understanding the factors that cause DNA degradation is critical to forensic science research and to understanding its effects on forensic investigations (1).

4. Literature Review

Effective preservation and analysis of DNA from bloodstains is critical to forensic science. While prior studies have advanced DNA extraction methods and identified factors influencing degradation, most have examined these variables in isolation. They focused on storage conditions, surface types, or temperature effects independently. Few have considered how these elements interact, which are frequently encountered in forensic contexts.

Several foundational studies inform this research. Shams et al. introduced a rapid, cost-effective method for extracting high-quality DNA from blood in various physical states: fresh, frozen, dried, and clotted. Their protocol minimized contamination and PCR inhibitors but was tested only under controlled laboratory conditions (2). Storage conditions also play a significant role in DNA preservation. The study showed that refrigerated EDTA-treated blood samples maintained high DNA quality, while room-temperature samples degraded more quickly (2).

Surface type and environmental exposure were studied by Sliskovic et al. who found that both sunlight duration and the nature of the surface significantly impacted DNA recovery from bloodstains. Their research highlighted that metal surfaces preserved DNA better than porous materials, yet they did not include fabric textiles (3). Further, Randa et al. demonstrated that high temperatures and burning significantly reduced DNA yields, although successful amplification was sometimes still possible. Their work suggests that even under extreme conditions, partial DNA preservation may occur. This aligns with the hypothesis that certain textiles may offer a degree of protection to biological material, enabling analysis even after prolonged environmental exposure (4). Together, these studies highlight the complex nature of DNA degradation. Yet, a notable gap persists in understanding how substrate material interacts with environmental variables in real-world conditions.

5. Research Design and Methodology

5.1 Experimental Design

The blood samples were collected using a lancet and the minimal volume required was obtained from the researcher. The University Institutional Review Board (UIRB) determined the study did not require approval. The clothing used for this experiment came from a local store, where 1 yard of fabric was obtained for each fiber: faux leather, wool, cotton, and polyester. Each yard of fabric was separated into 24, 3 x 5 squares. 50 µl of swine blood will be pipetted to each square and left in their designated environment dependent on the phase of the experiment. Phase 1 includes extraction and analysis of blood left in a petri dish over 1hrs, 24hrs, and 72hrs. Phase 2 includes extraction and analysis of blood left on the 4 different fibers for 1hrs, 24hrs, and 72hrs at room temperature. Phase 3 includes extraction and analysis of blood left on 4 different fibers

and left in 4 different environments for 1hrs, 24hrs, and 72 hrs. After each phase the DNA will be extracted from the square fibers using a phenol-chloroform extraction method. Once extracted, these sections of DNA will be analyzed for quality assessment using Agarose Gel Electrophoresis and Nanodrops.

5.1.1 Phase 1

To analyze how DNA in blood reacts in a controlled environment, phase 1 of the experiment was conducted. In this phase, 50µl of swine blood will be pipetted into four sterile petri dishes. These blood samples will be left at room temperature, and each petri dish represents a different incubation time. Those incubation times are 1hrs, 24hrs, and 72 hrs. After their allotted incubation period a phenol-chloroform extraction method will be used to extract DNA from the blood sample. The steps for the phenol-chloroform extraction method are as followed in the New England Biolabs Kit (5).

5.1.2 Phase 2

To analyze how the quality of DNA is affected when the samples are left on varying clothing fibers phase 2 must be followed. In phase two, 50µl of swine blood will be added to each clothing fiber: faux leather, wool, cotton, and polyester. In total there are 16 square fibers with 4 of each material. There will be 3 incubation times: 1hr, 24hrs, and 72hrs. After the incubation period DNA is extracted from the blood using the phenol-chloroform extraction method.

5.1.3 Phase 3

To compare how environmental changes affect clothing fibers and, in turn, impact DNA quality phase 3 must be performed. There will be 16 total square fibers with 4 of each material. 50µl of swine blood will be pipetted onto each square and each square will represent the three incubation periods. There will be three total treatment groups that have 16 square fibers each, and the groups will have different environmental conditions. Group 1 will have incubation periods of 1hr, 24hrs, and 72hrs at 130 °F. Group 2, will have incubation periods of 1hr, 24hrs, and 72 hrs at 0 °F. Group 4 will have incubation periods of 1hr, 24hrs, and 72 hrs while the fibers experience constant humidity. After their allotted incubation period the fibers will be dried thoroughly and using the phenol-chloroform extraction method the DNA will be collected.

5.2. DNA Analysis

After the DNA extraction further analysis will be conducted to determine the quality of the sample. The methods will be Nanodrop and Agarose Gel Electrophoresis.

5.2.1 NanoDrop

A NanoDrop is a spectrophotometer used to measure the concentration and purity of nucleic acids (DNA, RNA). It requires a small sample volume (typically 1–2 µL) and provides rapid, accurate readings. The program determines DNA Quality by measuring the absorbance of the sample at specific wavelengths. For DNA samples a 260 nm wavelength is used. Protocol for using a NanoDrop:

1. Turn on the NanoDrop and open the accompanying software.
2. Select the nucleic acid measurement mode (e.g., DNA, RNA).
3. Clean the pedestal with a kimwipe
4. Blank the instrument by loading 1 µL deionized water

5. Load the DNA sample (1 µL) onto the pedestal and lower the arm.
6. Measure the sample, and the software will display concentration and purity ratios.
7. Wipe the pedestal with a clean wipe after each measurement.

5.2.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis is a technique used to separate, visualize, and analyze DNA, RNA, or proteins based on their size. It works by applying an electric field to a gel matrix, causing negatively charged nucleic acids to migrate toward the positive electrode. Smaller fragments move faster and travel farther than larger ones. It determines DNA quality based on the integrity, purity, and size of the DNA. The protocol for Agarose Gel Electrophoresis (2).

6. Preliminary Implications

This research has the potential to extend current forensic science knowledge by providing a more nuanced understanding on how environmental conditions interact with different textiles to affect DNA degradation. While forensic studies have extensively examined DNA degradation over time, on different surfaces, and in different environments, few have considered the impact all these factors have on one another. This research has the potential to improve forensic interpretations of bloodstains on fabrics. If the study finds that certain textiles preserve DNA better in specific conditions, forensic investigators can develop more informed protocols for evidence collection and analysis. Overall, this study will contribute to forensic science by addressing an existing knowledge gap and laying the groundwork for future research on how material properties influence DNA degradation in real-world conditions.

9. Budget

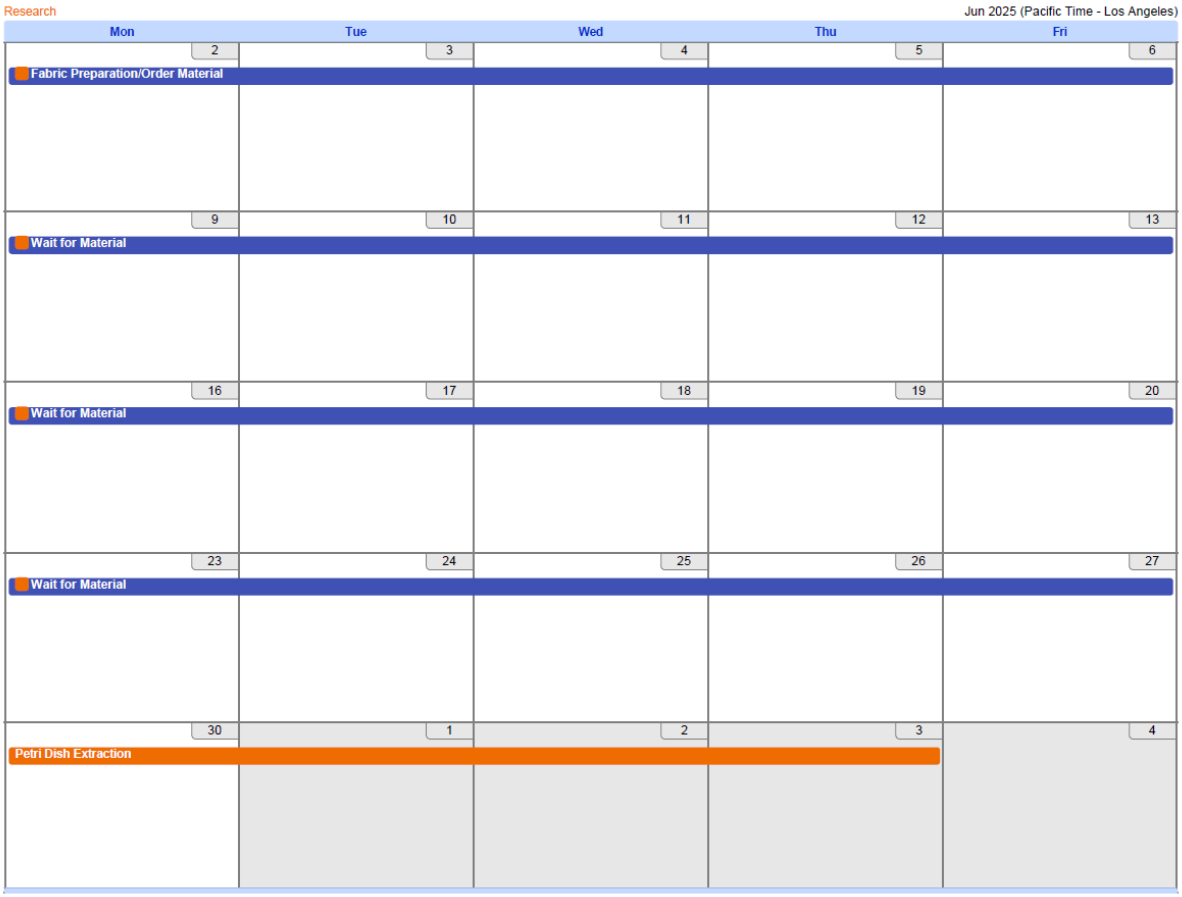
Table for Fabric/Extraction Material

Reagent / Component	Volume per Extraction	Total Volume for 51 Extractions	Approx. Cost (USD)
Fabric			$40
Tris-HCl (1 M, pH 8.0)	0.5 mL	25.5 mL	$25
EDTA (0.5 M)	0.5 mL	25.5 mL	$15
SDS (10%)	0.5 mL	25.5 mL	$25
Proteinase K (20 mg/mL)	0.05 mL	2.55 mL	$50
Phenol:Chloroform:Isoa	1 mL	51 mL	$100
Chloroform:Isoamyl Alcc	1 mL	51 mL	$30
RNase A (10 mg/mL, opt	0.01 mL	0.51 mL	$30
Isopropanol (100%)	1 mL	51 mL	$15
TE buffer or Nuclease-Fr	0.1 mL	5.1 mL	$10
			$410

Table for Agarose Gel Electrophoresis Reagents

Reagent / Item	Usage per Gel	Total for 51 Gels	Approx. Cost (USD)
Agarose (molecular biology grade)	0.5 g (1% in 50 mL)	25.5 g	$35 (for 100 g)
TAE Buffer (50X concentrate)	100 mL (1X per run)	~5.1 L (1X)	$30 (makes 10L 1X)
Gel Loading Dye (6X)	1 µL per sample	~1 mL (100 samples)	$15 (1 mL)
DNA Stain (GelRed/SYBR Safe)	2–5 µL per gel	102–255 µL total	$40 (500 µL)
DNA Ladder (1 kb or similar)	5 µL per gel	255 µL	$50 (500 µL)
Disposable Pipette Tips	~10 tips per gel	~510 tips	$20 (1,000 tips)
Microcentrifuge Tubes	~2 per sample	~102 tubes	$10 (500 tubes)
			$200

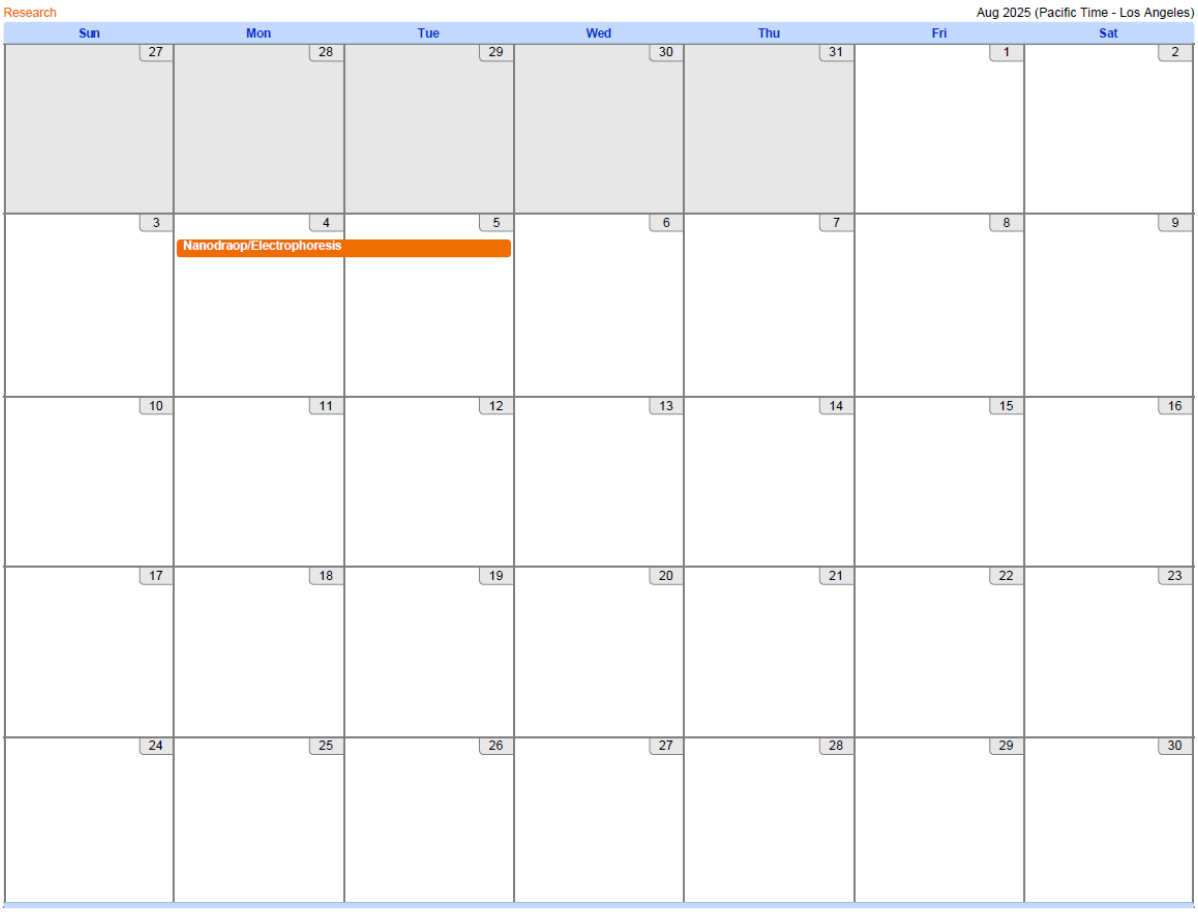
8. Hypothetical Timeline



Research

Jul 2025 (Pacific Time - Los Angeles)

Sun	Mon	Tue	Wed	Thu	Fri	Sat
29	30 Petri Dish Extraction	1	2	3	4	5
6	7 Fabric Extraction	8	9	10	11	12
13	14 Environment 1: Extraction	15	16	17	18	19
20	21 Environment 2: Extraction	22	23	24	25	26
27	28 Environment 3: Extraction	29	30	31	1	2



9. Conclusion

Many forensic science investigations rely on DNA evidence to solve and prosecute a criminal case. The integrity and preservation of DNA evidence is critical to the success of these investigations. Factors such as intense environmental exposer and material composition can significantly influence the rate of DNA degradation. This limits the reliability of forensic analysis thus harming the overall investigation. This research would address an important gap in forensic science research by investigating how different textiles and environmental conditions affect the degradation and extraction of DNA from bloodstains. Using a multi-step experiment and several DNA analysis methods this study will provide valuable insights into the interactions between environmental stressors and fabric material. The findings of this experiment have the potential to improve forensic collection protocols and guide interpretations of degraded samples; strengthening the reliability of forensic DNA analysis.

8. Citations

1. Bhoyar L, Mehar P, Chavali K. 2024. An overview of DNA degradation and its implications in forensic caseworks. Egypt J Forensic Sci 14:15.

2. Shams SS, Zununi Vahed S, Soltanzad F, Kafil V, Barzegari A, Atashpaz S, Barar J. 2011. Highly Effective DNA Extraction Method from Fresh, Frozen, Dried and Clotted Blood Samples. BioImpacts; ISSN 2228-5660 https://doi.org/10.5681/BI.2011.025.

3. Sliskovic L, Milos I, Zecic A, Kuret S, Sutlovic D. 2024. Does Sunlight Affect the Quality for Purposes of DNA Analysis of Blood Stain Evidence Collected from Different Surfaces? Genes 15:888.

4. Hady RHA, Thabet HZ, Ebrahem NE, Yassa HA. 2021. Thermal Effects on DNA Degradation in Blood and Seminal Stains: Forensic View. Academic Forensic Pathology 11:7.

5. Protocol for Extraction and Purification of Genomic DNA from Blood (NEB #T3010) | NEB. https://www.neb.com/en-us/protocols/2018/10/24/protocol-for-extraction-and-purification-of-genomic-dna-from-blood-t3010?srsltid=AfmBOopUskg5k201_FSyEz6KUfU8q5k7W4VEbIQGumIj3m78CC61r6E6. Retrieved 17 March 2025.