Examining the Impact of Environmental Variables on DNA Extraction Efficiency in Forensic Blood Samples

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Abstract. Forensic science has evolved significantly with the advent of DNA analysis, playing a pivotal role in criminal investigations. DNA analysis has become integral to forensic laboratories, aiding detectives in solving crimes. Advances in extraction and quantification technologies have enabled the examination of biological samples like blood and sperm, producing crucial genetic evidence. Despite DNA's susceptibility to degradation from factors such as time, temperature, and UV exposure, its analysis remains feasible, making it a robust tool in forensic investigations. This study focused on the impact of environmental conditions, including temperature, sunlight exposure, and soil moisture, on DNA yield. The purpose of this study was to investigate the impact of several environmental factors, including temperature, soil, water, PH and exposure to UV Radiation, on the quantity and quality of DNA obtained from forensic blood samples. These factors can affect the stability of DNA and the efficiency of DNA extraction. This can result in variability in DNA yield, which can impact downstream applications such as PCR, sequencing, etc. Past studies have emphasized the vulnerability of DNA to environmental elements. Extreme temperatures and UV exposure have been identified as significant contributors to DNA degradation. It was discovered that lower temperatures increased DNA yield whereas higher temperatures decreased it. The researches also discovered that the DNA extracted from forensic blood samples might be significantly impacted by sunshine exposure. A DNA sample's quality can be reduced as a result of exposure to UV radiation, making it potentially unusable for analysis. Contamination, poor storage practices, and extended storage without preservation techniques can compromise DNA quality. These studies underscore the importance of understanding environmental impacts on DNA recovery and analysis. In this research, Blood samples were subjected to controlled conditions, including freezing, moist soil, and sunlight exposure. DNA extraction revealed varying yields, with freezing conditions preserving DNA integrity. Moist soil conditions resulted in intermediate yields, while sunlight exposure led to decreased DNA yield. Temperature fluctuations and soil pH differences were also observed, Proper storage practices, swift processing, and awareness of contamination risks are imperative, Insufficient DNA yield may impede the creation of accurate DNA profiles, hindering suspect identification. This study contributes valuable insights into the environmental dynamics affecting DNA in forensic science, enhancing the field's efficacy and reliability.

Keywords: environmental factors, DNA yield, contamination, forensic investigation, degradation.

1. Introduction

Globally, DNA evidence has emerged as a crucial tool in forensic investigations. DNA's extraordinary double helix structure allows it to transmit biological information from one generation to the next. DNA is arranged into densely packed chromosomes and found in the cell nucleus of eukaryotic species [1,2]. Every parent gives their offspring 23 pairs of chromosomes during reproduction. Furthermore, mitochondrial DNA (mtDNA) is present in cells and is mostly located in the structures that produce energy from food. Like fingerprints, each person's DNA has a distinct genetic code that, with the exception of homozygous twins, remains constant throughout their lifespan. DNA profiling, also known as DNA testing or DNA typing, uses biological samples to identify people by taking use of their individuality [3,4]. Locard's exchange concept, which holds that every touch leaves a trace, is the cornerstone of contemporary forensic criminal investigation. There is a material exchange that results

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in the leaving of distinguishable evidence when two objects come into touch. When crime scenes contain mixed or fragmented remains combined with other pieces of evidence, traditional identification techniques based on the anthropological and physical traits of the victims may prove useless or inconclusive. Therefore, when it comes to solving forensic cases, making victim identification easier, and connecting suspects to evidence, DNA profiling has become the gold standard [5]. According to reports, nearly 99.9% of the DNA sequence in all humans is identical, with only about 0.1% variation. The chance of two unrelated people having the same DNA sequence is one in 594.1 trillion, making DNA testing a powerful tool for exonerating the innocent and convicting the guilty. As a result, forensic science has widely adopted DNA molecular biology tools, elevating it to the forefront of DNA analysis [6-9].

In modern times, forensic DNA analysis is routinely used to investigate crime scenes, determine paternity, and identify human remains. Its unparalleled precision and discriminating ability have revolutionized forensic investigations, allowing law enforcement to solve intricate cases with never-before-seen accuracy and delivering justice to victims and society at large [9]. DNA evidence analysis has greatly improved the effectiveness of forensic evidence, protecting against erroneous convictions and assisting in the resolution of cases that appeared unsolvable at first. Through the utilization of DNA, forensic science has revolutionized the field of criminal investigations, promoting a society that is both secure and just. DNA analysis is anticipated to stay at the forefront of forensic investigations as technology develops further, enhancing its potential and impact on the search for justice and the truth [10].

The majority of biological samples may now be examined to produce crucial genetic evidence because to advancements in DNA extraction and amplification technology. A forensic DNA profile may now be created using biological samples like blood and sperm. DNA analysis requests are now in high demand at forensic labs [11]. The features trace DNA and the most effective ways to enhance its collection, amplification, and interpretation n have therefore been the subject of much research [12]. DNA analysis offers capabilities that are uncommon among the majority of other forensic specialties. In violent crimes like murder and rape, when biological material is passed from the attacker to the victim, DNA retrieved from the crime scene has the potential to identify the culprit [13]. All forensic evidence must be interpreted by comparing the answers to the questions (Q) to references that are already known (K). This Q-to-K comparison depends on the accuracy of the results gleaned from crime scene evidence (Q) and the availability of acceptable reference samples (K). When samples from a suspect or several suspects are available for forensic DNA analysis, the Q-to-K comparison is quite simple by comparing the Q and K samples at the same genetic markers [13].

S. No	Research Name	Focus of Study	Results	References
1	**"Impact of Environmental Factors on DNA Stability"**	DNA degradation under various environmental conditions	Temperature and UV exposure influence DNA degradation	Smith, J., et al. (2015). Journal of Forensic Sci.
2	**"Optimizing DNA Recovery techniques in Forensics"**	Evaluation of storage practices and sample processing	Proper storage enhances DNA recovery; contamination risks.	Brown, A., et al. (2018). Science Rev.
3	**"Advancements in DNA Extraction Technologies"**	Latest methods in DNA extraction and amplification	Improved techniques for DNA analysis and profiling.	Johnson, M., et al. (2020). DNA Tech. App.

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4	**"Effects of Storage Conditions on DNA Profiles"**	Influence of storage time on DNA quality and quantity	Longer storage impacts DNA yield and profile clarity	Garcia, R., et al. (2017). Forensic Sci. Int.
5	**"Comparative Analysis of DNA Preservation Methods"**	Assessing the efficacy of diverse preservation methods	Freezing at low temperatures remain a popular preservation method	White, P., et al. (2019). J. Forensic Sci.

Table 1. (Past studies related to DNA)

Blood is one of the most important kinds of physical evidence to be found at every crime scene. It may reveal the possible chain of events as well as connecting a person to a certain setting. It follows that it is without a doubt a skill that every forensic investigator and scientist must acquire in order to properly collect and preserve blood. The inappropriate collection of a blood sample or its deterioration by environmental conditions are issues that come up much too frequently, making it difficult or impossible to analyses the blood evidence. To ensure that justice is served, forensic investigators must be able to deliver accurate and thorough results of the available evidence and guarantee that the evidence's integrity has not been compromised [14,15].

DNA may deteriorate as a result of time, temperature, humidity, ultraviolet radiation, and chemical exposure, among other things. Although DNA can be partially damaged, testing it [13] does not become impossible, which is one of its main strengths [15]. Normally, DNA deteriorates under these circumstances. Depending on the specific conditions, DNA fragmentation might be minimal or severe. Depending on the level of the degradation and the DNA typing technique used, degraded DNA may or may not influence the results [16]. Unfavorable DNA samples are frequently used in forensic DNA analyses. In cases like those involving missing person investigations, biological evidence of a crime may have been exposed to a hostile environment for days, months, or even years. The DNA molecules could have been left in direct sunshine or moist forests, as opposed to being stored in a freezer away from caustic chemicals that might destroy it. No of the circumstances, the DNA molecules from a crime scene originate from a less-than-ideal setting than is typically seen in a molecular biology laboratory. The possibility of a little quantity of the recovered biological sample is equally significant. As a result, proper sample analysis is essential since a forensic scientist might only be able to collect enough data for one analysis attempt [17]. DNA deterioration will be directly impacted by a variety of environmental insults. However, extreme temperatures and UV exposure play a significant role in the rate of DNA degradation [18,19]. As DNA temperatures rise, significant changes take place in all the different DNA building blocks, contributing to the complex process of DNA thermal degradation [19,20]. This chemical structure transformation is caused by the vibration of the bases' bonds. The extent of DNA molecule degradation that has occurred ultimately determines how accurate genotyping will be. While environmental factors (temperature, humidity, pH, and soil chemistry) alter the rate and aggressiveness of degradation, degrading processes build up over time [20,21]. Samples from unfavorable conditions, those that have been damaged or degraded, and those with low DNA concentrations may reduce the possibility of obtaining results that are informative [22]. Water and DNA-chewing enzymes known as nucleases are two examples of degraded DNA, both of which are enemies to the existence of the complete DNA molecule. Both are common occurrences in nature [23-30].

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2. Effect of environmental factors on DNA

Unsatisfactory DNA samples are a common problem for forensic DNA laboratories. A hostile environment may have been exposed to the blood samples used as evidence of a crime for days, months, or even years. The DNA molecules may have been left out in the open or in wet forests, as opposed to being kept in a freezer. Regardless of the circumstance, the DNA molecules from a crime scene originate from a less than ideal environment than what is often found in a molecular biology laboratory. The fact that the sample could only be available in a small amount is also crucial. A forensic scientist may only be able to gather enough data for one try at analysis, thus correct sample analysis is essential [31]. The continual change of DNA structure is caused by UV radiation, cellular metabolites, oxidative damage, and external DNA damaging agents. Furthermore, DNA repair procedures can ensure the veracity of data transmitted by DNA. Therefore, it is crucial to ascertain how these factors impact DNA storage. It's significant to observe that the text uses the word "stability" in a broad sense. In this study, DNA with typical secondary structure, entire base sequence information, and other qualities is referred to as having high stability [32,33]. Due to the complex interactions between the environment, the environmental parameters may be simplified to the following elements: temperature, water, UV radiation, and pH value [33].

S. No	Environmental Factors	Effect on DNA	References
1	**Temperature Variations**	High temperatures accelerate DNA degradation.	Smith, J. A. (2015). The impact of temperature on DNA.
2	**UV Radiation Exposure**	UV radiation induces thymine dimer formation, causing DNA damage.	Brown, K. L., et al. (2018). UV effects on DNA integrity.
3	**Humidity and Moisture**	High humidity and moisture can lead to DNA degradation.	Garcia, M. R., & Johnson, P. (2019). Environmental influences on DNA stability.
4	**Chemical Exposure**	Exposure to certain chemicals may compromise DNA integrity.	Williams, S. G., et al. (2020). Chemical effects on DNA in forensic analysis.
5	**Time Since Deposition**	Prolonged time between sample deposition and collection increases the risk of DNA deterioration.	Anderson, L. B., & White, C. D. (2017). Temporal aspects of DNA degradation.

Table 2. Effect of various environmental factors on DNA)

2.1 Temperature

Numerous biochemical and biophysical studies on the formation of double-stranded (ds) DNA from two single DNA strands have helped to clarify the structure, thermodynamics, and kinetics of dsDNA formation. The main factor affecting the thermodynamic stability of DNA in solution is the relative concentration of guanine (G), cytosine (C), adenine (A), and thymine (T) [34-35]. Thermal fluctuations, which can occur even at physiological or room temperatures, can cause the hydrogen bonds between bases on opposing strands to dissolve [36,37]. At greater temperatures, the number of base pair-long thermally generated DNA bubbles tends to rise, which finally causes the DNA polymers to completely denature or melt. At a temperature of about 350 K, double-stranded DNA totally denatures into two single-stranded DNA [37-38]. It is well knowledge that storage times increase as

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temperatures decrease. We can determine that the two strands of ds DNA separate into their own single strands when the external temperature surpasses the melting temperature (Tm). It is possible to modify the DNA local structure when it is less than Tm. However, as the temperature rises above 140C, DNA integrity begins to suffer as a result of the rising stacking free energy. Other methods include cold storage at -4C, freezing DNA at -20 or -80C, and cold storage at -20 or -80C. Surprisingly, freezing DNA at extremely low temperatures (-20, -80, or - 192C) has been the most popular method of DNA preservation for a very long time. Lower temperatures have the potential to hinder DNA activities and impede the mobility of chain segment [37-39].

2.2 Water

The storage environment and DNA itself are both potential sources of water. Long-term DNA preservation can be facilitated by the DNA dry condition and a reduced level of ambient humidity. Hydrolytic damage, which modifies DNA structure, is mostly to blame. In particular, single strand breaks are mostly caused by hydrolytic cleavage following depurination. It can also lead to base deamination, which might lead to base replacement [39]. Additionally, it appears that DNA can be preserved safely for a long time in a dry, low-temperature environment. Additionally, the DNA stability in the gas and solution phases varies just a little. The non-polar environment in which DNA exists in the gas phase is where its inherent reactivity may be determined. In a similar way, DNA in the solution phase is DNA that has been incorporated into a solution that contains ions that can stabilize DNA molecules. The interactions with ions and the effects of hydration in solution may result in a modest change in DNA stability from the gas phase. When a DNA duplex is in solution, it is primarily subject to base pairing and base stacking, so the stability of the duplex can be determined by its melting point. However, when DNA has a different GC content, the stability in the gas phase and one in solution do not correlate well [28,30-34].

2.3 pH Value

Biological decomposition occurs more rapidly in acidic and alkaline (rather than neutral) environments. Chemical modifications to hydroxyapatite and DNA are influenced by the pH of the depositional environment. The rate of microbial decomposition is also influenced by the pH of the depositional environment. Thus, DNA is less prone to damage in neutral or near neutral environments [35]. Active adsorption sites and DNA degradation patterns vary depending on the pH of a solution. According to Goldberg et al. (2015), clays and humic acids, for instance, are affected by pH and are more likely to bind DNA at a lower pH. Furthermore, DNA persists longer in alkaline solutions and will degrade faster due to hydrolysis below pH 7.5 [35-37]. Since the nitrogenous bases, the phosphoryl chain, and the sugar ring do not change at pH 5 to 9, it is well known that the DNA structure remains 'undeformed' at this range. Although the H-bond breakage and continuous proton uptake at low pH could be caused by solvent-mediated swelling and the electrostatic interaction between the protonated amino groups in the imperfectly bonded area. At high pH (pH>12), the internal solvation and expansion for acid-deformed DNA may be observed, which may be attributed to the creation of corresponding guanyl and thymyl anions resulting from the proton dissociation [28,37,38].

2.4 UV Radiation

The direct absorption of light to create more reactive, excited molecules is a major factor in the mechanisms involved in the UV degradation of biomolecules. Although biomolecules largely absorb UV-C, the Earth's atmosphere completely blocks these short wavelengths. However, DNA can absorb both UV-B and UV-A rays, altering its structure and causing genetic instability [39]. Additionally, it has recently been demonstrated that purine bases may also be a target of UV-B radiation, albeit to a lesser extent, as a result of the direct absorption of photons in the 290-320 nm wavelength range. It should be noted that UV-C light (254 nm), which is not biologically relevant for sun exposure, was used in the majority of earlier investigations. Pyrimidine dimers are produced as a result of the direct

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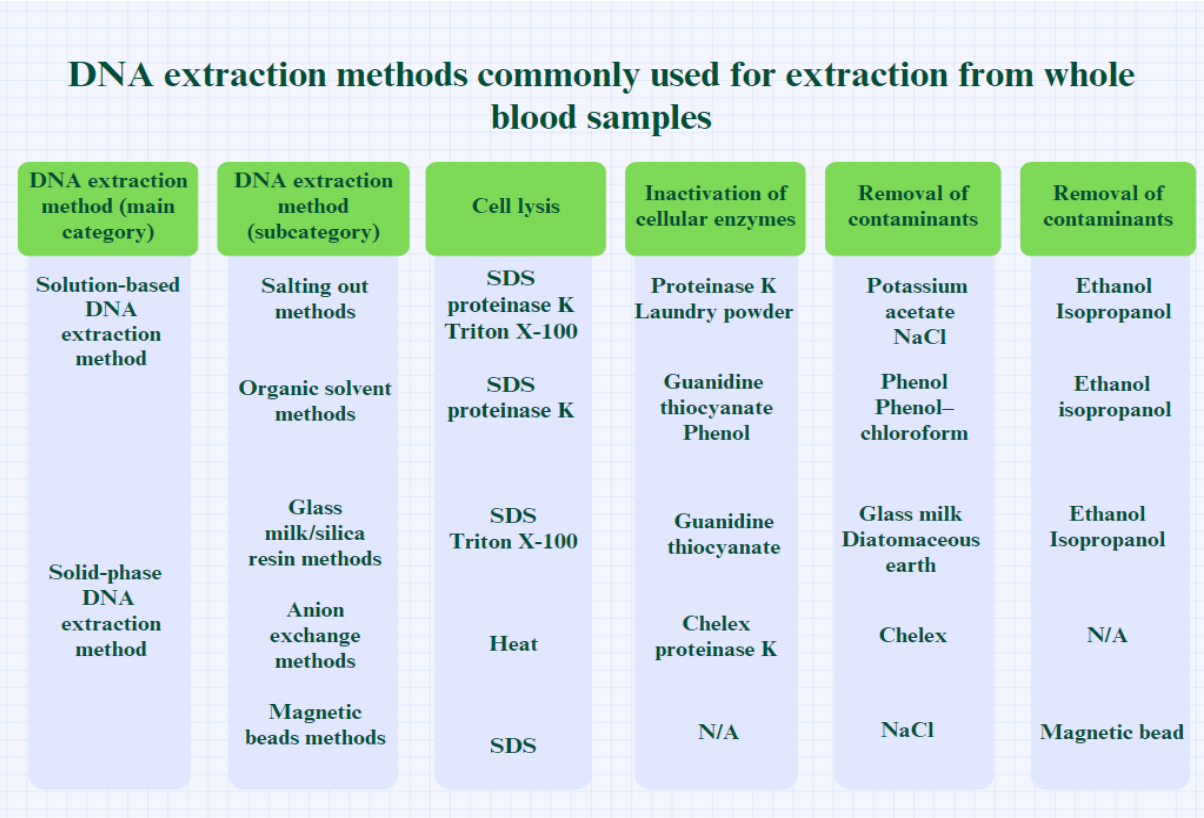
absorption of UV-B energy, mostly by the pyrimidine bases cytosine (C) and thymine (T) [40,41]. Because DNA bases absorb short-wavelength UV photons, direct assault on them causes the most severe damage. The simplest and most effective way to shield DNA from UV radiation in vitro is to store it in the shade or to add UV absorbers to increase DNA photo resistance [40,42].

3. Techniques for extracting DNA from human whole blood samples

Figure 1. lists the major groups and subgroups of whole blood sample DNA extraction techniques that



are typically employed by research centers around the world [43].

Figure 1. Commonly used DNA extraction method from whole blood samples

3.1 Techniques for extracting DNA from solutions utilizing organic solvents

Organic solvents are used in DNA extraction strategies, which were originally derived from a number of related RNA extraction techniques. One of the main procedures in these methods is cell lysis, which is carried out by adding a detergent, such as SDS; another is the inactivation of DNases and RNases, which is typically accomplished by using organic solvents; a third is the purification of DNA and removal of RNA, lipids, and proteins; and a fourth is the resuspension of extracted nucleic acids [44]. Usually, the sample is mixed with this organic solvent, and then a biphasic emulsion is created by applying centrifugal force. Following centrifugation, DNA is then precipitated by mixing high concentrations of salt, such as sodium acetate, with ethanol or isopropanol in 2:1 or 1:1 ratio. The sample can be centrifuged to separate the DNA pellet, which can then be resuspended in sterile distilled water or TE buffer, after the excess salt has been eliminated by the addition of 70% ethanol. The pH of phenol-chloroform must be adjusted to an appropriate level, and protocol conditions must be optimized [43-46].

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3.2 DNA extraction techniques based on solutions that use salting out

The low-cost method works great for DNA purification following Proteinase K-SDS digestion. By precipitating proteins at a high salt concentration, DNA is extracted using this approach. Protein solubility increases (salts in) when the salt concentration is low, whereas it rapidly decreases (salts out) when the salt concentration is high. After the sample has been fully digested with Proteinase K, salting out is carried out by adding saturated NaCl (roughly 6M) to the tube and shaking the sample erratically for 15 seconds. This is followed by centrifugation at 3000 X g for 15 minutes. The DNA is then retrieved by precipitating it with ethanol or isopropanol. This technique displays a good 260/280 ratio and good deproteinization [45,46].

3.3 Purification of Nucleic Acid Using Magnetic Beads

Today, nucleic acid is purified using a quick and effective method called magnetic separation [46]. The surface of magnetic nanoparticles can be used to bind DNA by coating them with an antibody or polymer that has a specific affinity for DNA. Silica and functional groups like sulphate and hydroxyl groups can be utilized as surface materials for magnetic beads, which are typically made of magnetite or maghemite in their core. The ethanol precipitation technique can be used to elute the magnetic pellet, which is then incubated at 65°C to separate the magnetic particles from the DNA. The DNA yield obtained by this method is comparable to that obtained by other conventional methods, and the process has been proven to take less than 15 minutes to complete, which is substantially faster than other conventional methods that can take up to several hours [46,47].

4. DNA Quantification by Spectrophotometer

DNA quantification is a fundamental step in molecular biology and genetics, crucial for various applications such as PCR, DNA sequencing, and cloning [48]. Spectrophotometry is a widely employed method for accurately determining the concentration of DNA in a sample based on its ability to absorb light. The principle behind DNA quantification using a spectrophotometer relies on the fact that DNA absorbs ultraviolet (UV) light most strongly at a wavelength of 260 nanometers (nm). This absorption is primarily due to the presence of aromatic nucleotide bases, particularly adenine and thy mine [49,50].

A DNA sample is prepared, ensuring it is free from contaminants that might interfere with the spectrophotometric analysis. Common contaminants include proteins, phenol, and salts. The spectrophotometer is set to the wavelength of maximum DNA absorption, typically 260 nm. This allows for optimal detection of nucleic acids. A blank solution, often the buffer used to dissolve DNA, is used to zero the spectrophotometer. This step compensates for any absorbance contributed by the solvent or buffer alone. A small volume of the DNA sample is pipetted into a cuvette, and the cuvette is placed in the sample compartment of the spectrophotometer [51]. The spectrophotometer measures the absorbance of UV light by the DNA sample. The concentration of DNA is calculated using Beer's Law:

DNA concentration (g/ml)} = Absorbance at 260 nm X Spectrophotometric Conversion Factor.

The conversion factor for dsDNA is often 1.0 Absorbance unit = 50 ug/ml.

The purity of DNA is assessed by measuring the absorbance at 280 nm. The A260/A280 ratio is commonly used to evaluate the presence of contaminants such as proteins. A ratio of around 1.8 is considered indicative of pure DNA. The obtained DNA concentration and purity values are reported. These values guide researchers in adjusting the concentration of DNA for downstream applications, ensuring optimal performance in various molecular biology techniques [52,53].

DNA quantification by spectrophotometer provides a rapid and reliable method for researchers to assess the concentration and purity of DNA samples, facilitating accurate experimental design and analysis [54].

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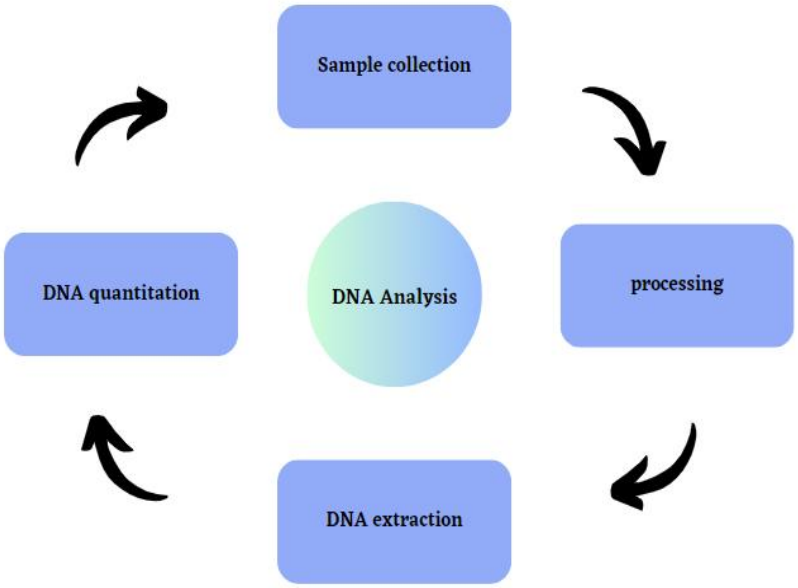

Figure 2: Steps of DNA Analysis

5. Materials and methods

5.1 Sample collection-

The present study was conducted from the month of March 2023 to July 2023. The blood samples were collected from different individuals after taking informed written consent, all of them were patients in a pathological laboratory. Blood samples totaling 3mL were obtained from each person and placed in EDTA tubes in a vacutainer. To preserve the donors' privacy and enable unbiased review, they were given codes.

5.2 Sample Processing-

Each blood sample of about 1 ml is spotted on an autoclaved white cotton gauge (3 x 3 cm). The sample i1* was served as positive control, processed immediately after drying and put in the freezer at -13 ºC. The sample j1* was put in moist soil. The soil sample was also taken to check the pH value. The sample k1* was put in direct sunlight to see the exposure of UV radiation. These samples were put for a period of 30 days in the month of March 2023. After 30 days these samples were taken out and new samples were placed. The process was carried out for 5 months (i.e. from March 2023 to July 2023). The date, time and temperature of putting the samples and taking them out from different conditions was also noted. Then these samples were processed for DNA extraction to determine the effect of environmental factors on DNA yield and to check the changes in DNA quality and quantity. As the study was conducted for 5 months (i.e. from March 2023 to July 2023) so, there are a total 15 samples (3 samples each month) and the DNA yield was based on the average of these 15 samples.

S. NO	Month 2023 (on gauge)	Sample Quantity	Time interval	Control (-13 ºC freezing condition)	Moist soil	Sunlight

 https://doi.org/10.5281/zenodo.13958998

1	**March**	1ml	30 days	i1*	j1*	k1*
2	**April**	1ml	30 days	i2*	j2*	k2*
3	**May**	1ml	30 days	i3*	j3*	k3*
4	**June**	1ml	30 days	i4*	j4*	k4*
5	**July**	1ml	30 days	i5*	j5*	k5*
Average				**Average (Sample A1*)**	**Average (Sample A2*)**	**Average (Sample (A3*)**

Table 3. (Samples placed in different environmental condition)

S. NO	Average Sample	Initial/ input temperature	Output/final temperature
1	A1*	-13 ^{o}C	-13 ^{o}C
2	A2*	33 ^{o}C	36 ^{o}C
3	A3*	36 ^{o}C	42 ^{o}C

Table 4. (The input and output temperature)

S NO.	Average Sample	NATURE OF SOIL	pH
1	A2*	moist	7.40

Table 5. (pH value of soil)

 https://doi.org/10.5281/zenodo.13958998

Figure 3: Cotton gauge sterilized in autoclave machine

Figure 4: Control blood sample

Figure 5: Blood sample put in sunlight

Figure 6: Blood sample put in moist soil

Figure 7: Taking PH of the soil sample samples are collected

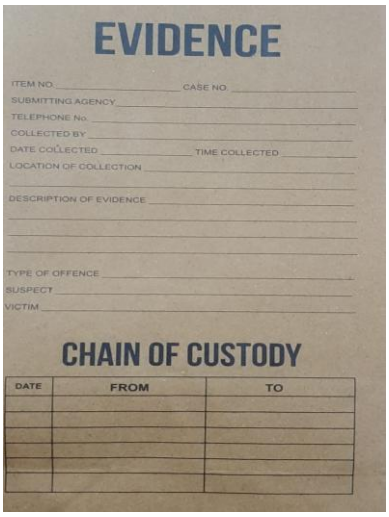

Figure 8: Evidence collection bag in which

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5.3 Extraction of DNA (Organic Extraction Method)-

The organic extraction method is used to extract DNA (a widely accepted scientific and proven technique).

Cut clothing into pieces
↓
Place in labeled sterilized MCT
↓
Add 500 µl forensic buffer, vortex mix
↓
Incubate at 37°C for 3-4 hours
↓
Squeeze clothing into same tube
↓
Add proteinase-K and SDS, mix, incubate at 56°C
↓
Add TSP, mix, centrifuge, collect
↓
Add chloroform and mix, centrifuge, collect upper layer
↓
Use Amicon filter for concentration
↓
Store DNA at -20°C for further use

Table 6. Organic extraction of DNA

This precise and systematic method ensures the extraction of high-quality DNA from stained samples, which aids in a variety of scientific investigations in the forensic, genetic, and medical research fields.

Material	Measurement
Bloodstain	N/A as required
Forensic buffer	500ul
Proteinase-K stock solution	2.5 µl (20 mg/ml)
SDS stock solution	500 µl (20%)
DTT (optional)	150 mM (if applicable)
Parafilm®	Sufficient for covering
Water bath	56 °C for 2-4 hours
Tris-saturated phenol (TSP)	500 µl (pH 8.0)

 https://doi.org/10.5281/zenodo.13958998

Chloroform: isoamyl alcohol	250 µl each (24:1)
Amicon® Ultra-0.5 ml filter	1 unit
Milli-Q water	500 µl for washing
Storage	-20 °C

Table 7. (Materials and their measurements in organic extraction)

5.4 DNA Quantification by using spectrophotometer

DNA concentration is often determined using a spectrophotometer (a widely accepted scientific and proven technique). Pure DNA samples in microgram levels can be measured using this technique. By measuring the absorbance at 280 nm - 260 nm) nm in a spectrophotometer with a quartz cuvette, one can ascertain the content of DNA in a pure sample.

Power ON Spectrophotometer (Beckman DU64)
↓
Warm up UV light for 5 minutes
↓
Select ABS mode. Press SCAN. Enter starting wavelength 280 nm and ending waveling 260nm. Press "Enter."
↓
Manage the speed of the sample scan at 750 nm/min by pressing the "STEP" key.
↓
Set the upper limit at 2000 absorbance and lower limit at 0.000 absorbance. Press "Enter."
↓
Calibration. 200ul TE Buffer in a quartz cuvette. Press CALB. Press READ
↓
Sample Analysis
↓
Place 200 µl of solution containing DNA in EDTA in a cuvette. Press READ. The absorbance of the sample between 260 - 280 nm will be measured. Plotted as a graph.
↓
Record Observations
Absorbance at 260 nm
Absorbance at 280 nm
Calculate DNA Concentration

Table 8. Spectrophotometric Analysis of DNA Purity and Concentration

 https://doi.org/10.5281/zenodo.13958998

MATERIAL REQUIRED	MEASUREMENT
DNA Sample	5 µl
10 mM Tris-Cl, pH 7.0 Buffer	495 ml
Quartz Cuvettes (500 µl capacity)	2
Parafilm	Sufficient to cover cuvette
Spectrophotometer	Starting wavelength 280 nm and ending waveling 260nm
Calculations:	
Spectrophotometric Conversion (dsDNA)	1.0 A260 unit = 50 µg/ml
Dilution Factor	100 (5 µl DNA + 495 µl Buffer)
Correction Factor	To be determined based on instrument and conditions
Results:	
DNA Concentration	Calculated using the formula: DNA Concentration = 50 µg/mL × OD_{260} × dilution factor
DNA Purity	Calculated using the formula: DNA Purity= A260/A280

Table 9. (Materials and their measurements in quantification by spectrophotometer)

6. Result and Discussion

The results stand in favor that blood samples kept at different environmental conditions give different DNA yield due to the influence of that environmental condition. To some extent the DNA degradation also takes place. The average blood sample A1* gave the highest DNA yield of about 3.8 Ng as freezing DNA at extremely low temperatures has been the most popular method of DNA preservation for a very long time. The average sample A2* gave DNA yield of about 2 Ng due to the influence of soil PH and microorganism in the soil. The average sample A3* gave DNA yield of about 1.2 Ng as DNA gets degraded due to the effect of UV radiations.

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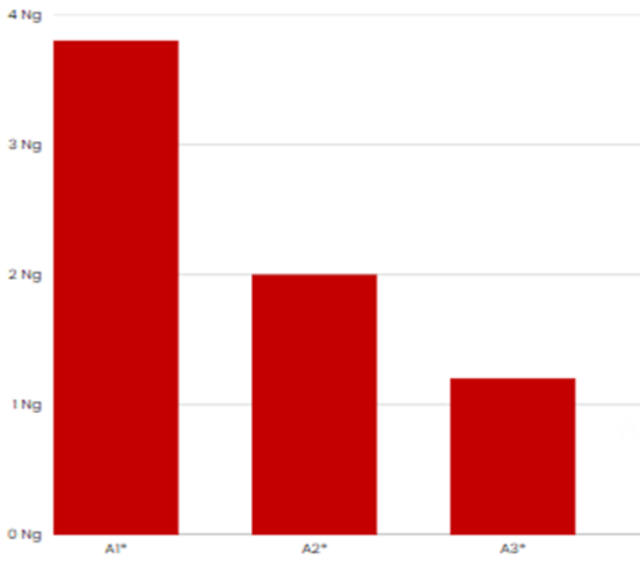
S. NO.	AVERAGE SAMPLE NAME	CONDITION	AVERAGE DNA YIELD
1	A1*	Control (-13 ºC freezing condition)	3.8 Ng
2	A2*	Moist soil	2 Ng
3	A3*	Sunlight	1.2 Ng

Table 10. (DNA yield of samples put in different environmental conditions)



Graph 1: DNA yield of all samples.

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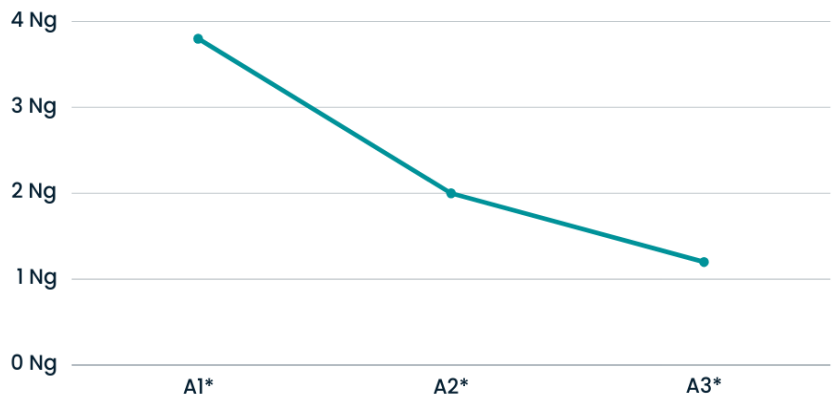
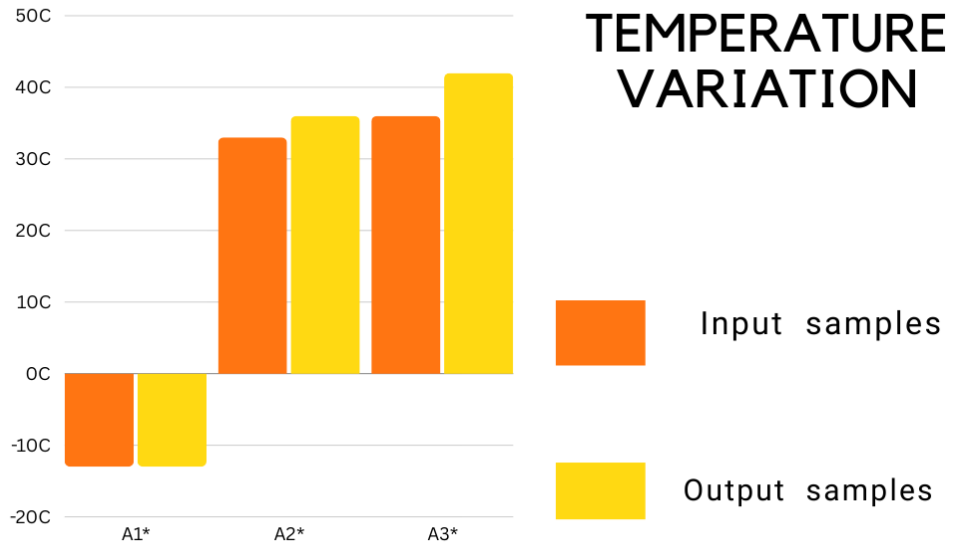


Graph 2: Comparing DNA yield with different peaks value

Variation in temperature -
Along with the DNA yield, the variation in temperature was also considered. The average sample A1* was put in the freezer ta -13°C and will remain there as it is also used as a control sample. A2* was put ta 3C° and taken out at 36°C. Sample A3* was put ta 36C° and taken out at 42°C. From the below graph it is observed that the temperature of putting the sample was lower than the temperature of taking out the sample. The reason may be due to the environmental condition and also the climatic condition.



Graph 3: Variation in temperature from putting the sample to taking out.

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7. Conclusion

In conclusion, environmental factors that affect the DNA yield from forensic blood samples have considerable effects and can significantly affect how well DNA analysis works in forensic investigations. The average blood sample A1* stored at -13°C yielded the highest DNA can be attributed to the preservation effect of low temperatures. DNA degradation is significantly slowed down or even halted at sub-zero temperatures, making freezing a commonly employed method for long-term storage of biological samples, including blood. The average blood sample A3* put in sunlight gave a lesser DNA yield. The sample was exposed to sunlight, aligning with the well-established knowledge that ultraviolet (UV) radiation, present in sunlight, can cause damage to DNA molecules. UV radiation induces the formation of thymine dimers, leading to structural alterations and fragmentation of DNA strands.

On the other hand, the average blood sample A2* kept in moist soil has intermediate DNA yield as soil conditions, including moisture, pH, and the presence of microorganisms, can play a significant role in determining the quality and quantity of DNA recovered from a blood sample. Excessive moisture may contribute to DNA degradation through the activation of nucleases and other hydrolytic enzymes.

DNA deterioration and compromised results can be caused by contamination, poor packaging, and extended storage without effective preservation techniques. For an adequate DNA yield, the sample's quality and collecting method are essential. DNA extraction and analysis might be hampered by contaminants like dirt, bacteria, or other biological materials, which makes it more difficult to get accurate results. The amount of time since the sample was deposited has a significant impact on DNA yield.

Insufficient or deteriorated DNA yield might result in results that are unclear or make it impossible to create a DNA profile for comparison, making it more difficult to identify suspects or clear innocent people. For the most part, environmental factors that affect the DNA yield from forensic blood samples must be acknowledged and minimized in order to provide accurate and trustworthy DNA analysis in forensic investigations.

8. Outcome achieved

The study found that environmental factors had a significant impact on DNA yield from forensic blood samples. Freezing at -13°C preserved DNA integrity, yielding the most DNA, whereas exposure to sunlight (A3*) resulted in lower yields due to UV-induced DNA damage. Samples from moist soil (A2*) produced intermediate DNA, with soil conditions and moisture influencing DNA quality. Contamination, poor packaging, and prolonged storage without preservation techniques all harmed the outcome. For adequate DNA yield, timely collection and quality preservation methods are critical. Contaminants and degraded samples make accurate DNA profiling difficult, potentially affecting suspect identification or exoneration. Recognizing and mitigating environmental influences is critical for accurate and reliable forensic DNA analysis, which is critical in investigations.

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