

# A comparative quantification of DNA from blood stains on canvas at different time intervals

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**Abstract:** In forensic investigations, bloodstains are essential biological evidence that helps with identification and event reconstruction. This study looks at the extraction and measurement of DNA from bloodstains that have been left on canvas surfaces across various time periods. A good paradigm for examining biological evidence behaviour is canvas, a porous cotton-based substrate. Following controlled aging of blood samples for 1<sup>st</sup> day to 20<sup>th</sup> day, DNA will be extracted using conventional phenol-chloroform techniques and quantified using UV-Spectrophotometer. Understanding time-dependent DNA degradation, which results in decreased DNA quantity and quality, is the main goal of the study. In order to improve forensic analysis and timeline estimation, the study attempts to establish a relationship between bloodstain age and DNA degradation patterns.

**Keywords** - DNA extraction, DNA quantification, bloodstains, Forensic science.

## INTRODUCTION

Bloodstains are an important source of biological evidence in forensic investigations because they can provide DNA for human identification and assist in crime scene reconstruction. However, the quality of DNA recovered from a bloodstain depends on how well it is preserved after deposition. Exposure to environmental factors such as temperature, humidity, ultraviolet (UV) radiation, oxygen, and microbial activity can gradually damage DNA, resulting in reduced quantity and integrity and making subsequent forensic analysis more difficult (Alaeddini, 2012; Butler, 2009). The substrate on which a bloodstain is deposited can also influence DNA preservation and recovery (Prinz & Berghaus, 1990; van Oorschot et al., 2019). Porous materials absorb blood into their fibrous structure, allowing cellular material to become trapped within the substrate. Canvas paper is a cotton-based, fibrous and porous material that can retain biological material within its fibre network. Such materials may be relevant to forensic investigations because blood can remain embedded in their structure for prolonged periods. However, only a small amount of study has looked at how DNA extracted from bloodstains explicitly placed on canvas surface has changed over time. Studying this behaviour may therefore provide useful information for the interpretation of aged biological evidence. As a bloodstain ages, environmental and biochemical processes may cause DNA molecules to break down into smaller fragments. According to Cossette et al. (2021), this degradation can lower the quantity of recoverable DNA and impact its compatibility for subsequent molecular analysis. A number of variables, such as storage conditions, exposure duration, and substrate properties, influence the degree of deterioration (Żarczyńska et al., 2023). Therefore, examining DNA recovery at defined time intervals can help in understanding the persistence of biological evidence under controlled conditions (Stotesbury et al., 2021). Efficient DNA extraction is essential for obtaining usable genetic material from bloodstains. Several extraction methods are available, including organic extraction, Chelex-based extraction, silica-based methods, and automated systems. In the present study, the phenol–chloroform organic extraction method was used because it enables effective cell lysis, protein removal, and recovery of genomic DNA from biological stains. The extracted DNA was subsequently quantified using UV spectrophotometry by measuring absorbance at 260 and 280 nm. The A<sub>260</sub>/A<sub>280</sub> ratio was used as an indicator of DNA purity and possible protein contamination (Wilfinger et al., 1997). DNA profiling has become a fundamental tool in forensic science for human identification. It primarily relies on

highly variable genetic regions, which provide considerable discrimination between individuals. The reliability of DNA profiling, however, is influenced by the quantity and quality of DNA recovered from biological evidence. Consequently, understanding how these characteristics change as a bloodstain ages is important for the interpretation of forensic samples.

## NEED OF THE STUDY

The present study was designed to evaluate changes in DNA recovered from bloodstains deposited on canvas surface at different time intervals under controlled conditions. DNA was extracted using the phenol-chloroform method and assessed through UV spectrophotometric analysis to determine changes in DNA concentration and purity. The study aims to provide a better understanding of time-dependent changes in DNA recovered from a porous cotton-based substrate and to contribute to the interpretation and evaluation of aged bloodstain evidence in forensic investigations.

## METHODOLOGY

In this research, DNA was isolated from bloodstains on canvas, which is a porous cellulose-derived substrate. At each designated storage time, a 2 mm section of the bloodstained canvas was aseptically cut with sterile scissors and put into a sterile microcentrifuge tube. DNA was extracted from the bloodstained canvas samples using the phenol–chloroform organic extraction method, which is renowned for its ability to recover high-quality genomic DNA from forensic biological evidence. (Nguyen Thi Hue, T., *et al.* (2011). Each sample was processed with 400 µL of lysis buffer and Proteinase K, followed by incubation at 56°C for 3 hours to ensure complete cell lysis. (NIJ,n.d.) DNA training literature expressly mentions that organic extraction can be utilized for bloodstains, with lysis buffer + Proteinase K followed by phenol/chloroform/isoamyl alcohol extraction. Older samples were incubated overnight to optimize DNA recovery. After lysis, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, and the samples were mixed thoroughly before centrifugation at 10,000 rpm for 10 minutes. After carefully transferring the DNA-containing upper aqueous phase to a new tube, it was purified using chloroform:isoamyl alcohol (24:1). 3 M sodium acetate and cold 100% ethanol were added to precipitate DNA. To create a DNA pellet, the mixture was centrifuged at 12,000 rpm for 15 minutes after being incubated at a low temperature for 30 to 60 minutes. For DNA quantification and further analysis, the pellet was cleaned with 70% ethanol, allowed to air dry for ten minutes, and then dissolved in TE buffer.

### 3.1 DNA Extraction

DNA was extracted from bloodstains deposited on canvas using the phenol-chloroform organic extraction method (Köchl *et al.*,2005; Butler, 2012). Sterile scissors were used to cut about 2 mm of the stained canvas, which was then put into sterile microcentrifuge tubes. Each sample was processed with 400 µL of lysis buffer and Proteinase K, followed by incubation at 56°C for 3 hours to ensure complete cell lysis. Older samples were incubated overnight to optimize DNA recovery. After lysis, the materials were thoroughly mixed with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and centrifuged for 10 minutes at 10,000 rpm. Without upsetting the interphase, the DNA-containing upper aqueous phase was gently moved to a new tube. Chloroform:Isoamyl alcohol (24:1) was used in a second purification step, which was then centrifuged for five minutes at 10,000 rpm. 3 M sodium acetate and cold 100% ethanol were added to precipitate DNA. To create a DNA pellet, the samples were centrifuged at 12,000 rpm for 15 minutes after being incubated at a low temperature for 60 minutes. Before being dissolved in nuclease-free water for further examination, the pellet was cleaned with 70% ethanol and allowed to air dry for ten minutes.

### 3.2 DNA Quantification

A UV spectrophotometer was used to measure the extracted DNA's concentration and purity. Absorbance at 260 nm was used to estimate DNA content, and the A260/A280 ratio which shows potential protein or other impurity contamination was used to evaluate purity (Shen, 2023). DNA quantification was used to determine if each sample was suitable for subsequent molecular analyzes and to determine how storage time affected the recovery of DNA from bloodstains on canvas surfaces (Lee et al., 2014).

### 3.3 Data Recording and Analysis

Each sample's DNA content ( $\mu\text{g/mL}$ ) and purity ( $A_{260}/A_{280}$ ) values were methodically recorded and categorized based on the corresponding storage intervals. To assess changes in DNA quantity and quality over time, mean values were computed for each interval. To make it easier to compare the data over various aging periods, tables and graphical representations were used. In order to evaluate DNA degradation and their forensic significance in determining the amount of time since bloodstains were deposited on porous substrates, observed trends were analyzed.

## RESULTS AND DISCUSSION

As storage time increased, the bloodstains gradually changed in colour and appearance. Overall, as the storage period lengthened, gradual illumination and bloodstain drying were noted. Visual Appearance and Colour Changes of Bloodstains on Canvas. During Storage: First Day (Dark reddish): A new stain with uniform appearance and clearly defined borders. Day 5 (light reddish): The stain seems drier and less vivid, and there is a discernible decrease of red pigment. Day 10 (Light reddish-brown): The oxidation and drying of blood pigments causes the colour to change to a light reddish-brown. 15th Day (Light brown): The stain appears light brown with a little fading. The stain looks entirely dried and aged on the twentieth day (light brownish), when the aging effects are at their peak.

Average DNA Concentration - The average concentration of DNA clearly decreased as storage duration increased. After the first day, the mean concentration was the highest ( $88.4 \mu\text{g/ml}$ ), and after the twentieth day, it was the lowest ( $36.0 \mu\text{g/ml}$ ). Overall, the findings show that the concentration of recoverable DNA gradually decreased as the storage period lengthened, indicating a time-dependent loss of DNA from bloodstains left on canvas paper. Over the course of the storage period, a steady decrease in DNA concentration was noted. DNA concentration dropped by 16.74% after the fifth day in comparison to the first-day samples, and it reached a maximum fall of 59.28% after the twentieth day. Measurable amounts of DNA were successfully recovered from every storage interval despite this reduction.

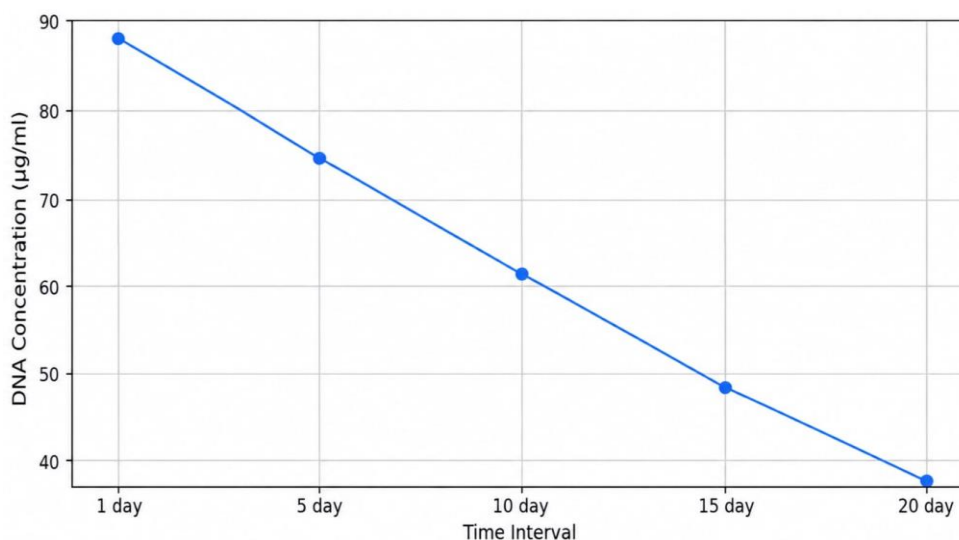


Fig. 4.1: DNA Concentration vs. Time graph.

DNA Purity ( $A_{260}/A_{280}$  Ratio) at Different Time Intervals - DNA purity was assessed using the  $A_{260}/A_{280}$  absorbance ratio. Pure DNA typically exhibits an absorbance ratio close to 1.8. (Thermo Fisher Scientific, 2017). To assess DNA purity, the  $A_{260}/A_{280}$  ratio was tracked over a 20-day storage period across five replicates per time point; Day 1: With ratios closely falling between 1.81 and 1.85, the samples showed ideal DNA purity, Day 5: Purity ratios showed a small decrease, falling to a range of 1.76 to 1.80, Day 10: The ratios showed signs of early breakdown or mild protein contamination as they continued to decline and fell into a range of 1.70 to 1.74, Day 15: Ratios ranging from 1.63 to 1.67 showed a more noticeable decline in purity, Day 20: The final time point showed the lowest purity levels, with ratios falling to a range of 1.56 to 1.60. All things considered, the data shows a consistent, time-dependent decline in the  $A_{260}/A_{280}$ .

Average purity as a storage time increases, the A260/A280 ratio gradually changes. In the first-day samples, the values stayed near 1.8, suggesting good DNA purity. The following intervals showed a consistent decline, with mean values of 1.78, 1.72, 1.65, and 1.58 on the fifth, tenth, fifteenth, and twentieth days, respectively. After the twentieth day, the ratio was at its lowest. Overall, the findings show that when the bloodstains were stored on canvas for an extended period of time, the DNA purity gradually declined.

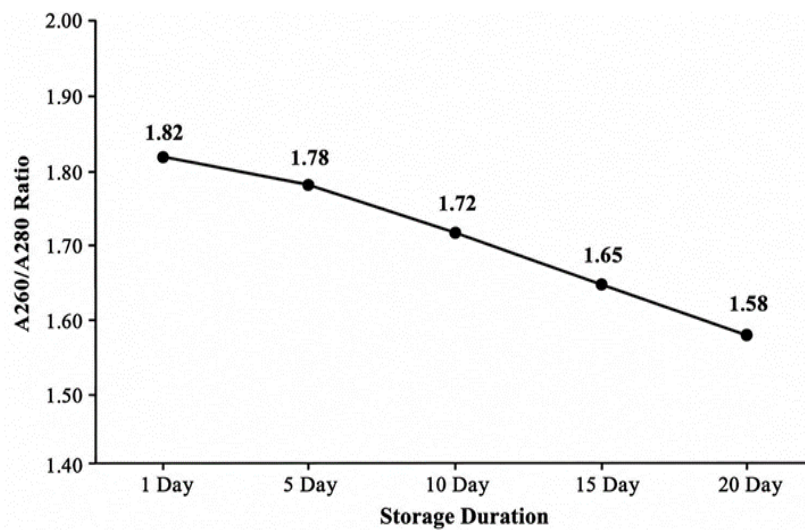


Fig. 4.2: DNA Purity vs. Time graph

The findings demonstrated that DNA purity gradually decreased while being stored. The highest average purity ratio (1.82) was found in bloodstains analyzed after the first day, indicating comparatively pure DNA. As storage time increased, the ratio gradually decreased and reached 1.58 after the twentieth day. This decline points to increasing DNA deterioration and lower sample quality during extended storage. Nonetheless, DNA was still identifiable during the research period, suggesting that even after the twentieth day, bloodstains left on canvas paper had recoverable genetic material.

The current investigation showed that the length of storage had a major impact on the quality of retrieved DNA as well as the physical look of bloodstains. While DNA concentration and purity steadily decreased over time, bloodstains grew increasingly darker. Measurable DNA was retrieved from every sample, despite significant reductions seen after the 20th day of preservation, demonstrating the potential forensic usefulness of old bloodstains left on canvas surfaces.

The findings indicated that as storage time increased, both DNA concentration and A260/A280 purity gradually decreased. Samples taken on the first day had the highest DNA concentration and purity, whereas samples taken after the twentieth day had the lowest values. This decrease could be the result of DNA gradually deteriorating during storage due to oxidation, drying, and other environmental conditions. All bloodstained canvas samples, including those kept for 20 days, had their DNA effectively extracted. Canvas porosity structure might have contributed to the blood's retention inside its fibers, making DNA recoverable over time. The decline in DNA concentration and purity, however, suggests that the amount and quality of recoverable DNA may be impacted by extended storage.

Overall, the results show that the amount and quality of DNA from bloodstains left on canvas decrease with time. The study also emphasizes how crucial it is to gather biological evidence promptly and preserve it properly in order to improve DNA recovery for forensic examination.

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