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Study on Persistence and Detection of Blood Stain DNA on Pig Skin Exposed to Different Aquatic Conditions

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Abstract:

It's a common practice for perpetrators, washing blood stains with different types of water and detergents, to remove blood evidence and traces at the scene of the crime. Multiple studies carried out around the world have shown that DNA is present in washed blood stains, which show that protective blood cell structures are often able to shield them from degradation of DNA. In this study, we used pig skin, smudged with mice blood, and were kept in four different containers labelled as fresh water, salt water, fresh water with detergent and salt water with detergent with variable pH condition for 6 hours, 12 hours and 18 hours to check the persistence of DNA. A total number of 12 samples were used. All in all, the results demonstrate that specifically those samples which was immersed in fresh water, was possible for extraction of DNA. The samples which were immersed in other aquatic conditions was not viable for extraction of DNA. As detergents contain enzymes that are designed to break down biological molecules including DNA and increased pH in these systems enables DNA to get denatured faster compared to salt water and fresh water system.

Keywords: *DNA, Persistence, Aquatic condition, Immersed sample, Forensic, Detection*

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Introduction

Identifying and determining the origin of blood stains in various crimes forms important physical evidence. Forensic scientists must examine every type of material recovered under many different conditions for the purpose of determining whether blood is present **(Jain and Singh, 1984)**. Water is an important factor affecting DNA integrity **(Frippiat *et al.*, 2017)**. Forensic evidence from blood is highly valuable and has been a determining factor for solving many criminal cases **(Wickenheiser, 2002)**.

The presence of a bloodstain is primarily observed as biological evidence at the crime scene, particularly in violent crimes **(Sapan *et al.*, 2021)**. Touch DNA and Trace DNA is the type of DNA commonly found in the crime scenes and the most missed evidence. The amount of DNA transferred is affected by numerous factors, some of which may be intrinsic to the individual, such as shedder status **(Lowe *et al.*, 2002)**. Temperature extremes affect the degradation profile of bloodstains **(Cossette *et al.*, 2021)**. The cleaning agents lead to the degradation of DNA and also cause changes in DNA structure. The DNA double strand will also get affected by the influence of detergent **(Judah *et al.*, 2023).**

Examining bloodstains is of immense value in reconstructing a crime scene and linking the perpetrator or victim to the crime scene. criminals today often try to clean up the crime scene, and it is not known under what conditions the bloodstain passed before the analysis. The detection of bloodstains can be done with the help of luminol as it does not have any destructive effect on the extraction of DNA as well on the blood test **(Passi *et al.*, 2012).** The aim of the study was to extract the DNA from the pig skin after been kept in a particular aquatic sample (i.e. fresh water, Salt water, fresh water with detergent & Salt water with detergent) for 6, 12 & 18 hours.

Material and Method

- **Samples**

Hairless pig skin samples were obtained from young pigs from the butcher shop. The skin was cut into 5 mm thickness and 2 * 2 cm squares dimensions (n=12) to test persistence of DNA. The specimens were washed with DNA-free water and stored in the freezer until the experiments were conducted. Four different aquatic conditions were taken fresh water, salt water, fresh water with detergent and salt water with detergent. Fresh mice blood was taken for the experiments and was smudged into each sample (n=12).

- **Set-up**

For each aquatic conditions, three containers were taken to check the persistence of DNA in 3 different time span i.e. 6 hours, 12 hours and 18 hours respectively. The samples had been immersed on each of the four aquatic conditions.

- **2.3 DNA Extraction**

Ethanol precipitation is commonly used technique for extracting nuclei acid (DNA). Took each sample and kept in sodium acetate salt (0.03 M) and ethanol to force out the precipitation of nucleic acid in a pellet form. Later, the pellets are washed with 70% ethanol and the pellets are allowed to dry **(www.Bitesizebio.com).**

Results

Table No. 1: Positive and negative observation of DNA extraction after the sample immersed in aquatic conditions.

Aquatic Conditions	6 hours	12 hours	18 hours
Fresh water (FW)	+	+	-
Salt water (SW)	+	-	-
FW + detergent	+	-	-
SW + detergent	-	-	-

From the above table, we observed that DNA is found persistent up to 12hrs and viable for extraction in case of fresh water and the same is found up to 6hrs in case of salt water but DNA had been completed denatured and found non-viable for extraction in both aquatic systems mixed with detergents because of fact that detergents contain enzymes that are designed to break down biological molecules including DNA and increased pH in these systems enables DNA to get denatured faster compared to salt water and fresh water system.

Discussion

To determine the utility of evidence-based DNA analysis, we examined DNA preservation in four different situations corresponding to actual casework: blood in fresh water, blood in salt water, blood with fresh water and detergent, and blood with salt water

and detergent. The pig skin closely resembles the human skin from both histological and physiological aspects **(Uhm *et al.*, 2023).** Almost all of the genes in mice share functions with the genes in humans **(www.jax.org).** After a criminal act, a perpetrator would try washing bloodstains **(Sapan *et al.*, 2021).** Cases are unrecorded due to environmental conditions or society limits where people don't report the crime for weeks which leads to loss of evidences.

Limitation

1. Maintenance of possible time sensitivity for longer duration with controlled conditions.

2. Mice blood as an alternate for human blood in the study.

3. Unavailability of quantification methods like STR and PCR based DNA analysis for accurate information.

4. Variable pH, water hardness and microbial population affects the results and hence the results cannot be considered generic and instead it can be used in terms of controlled conditions.

Conclusion

In this initial experimental proof-of-concept study, we investigated the persistence of blood DNA in skin samples immersed in water. Our results showed that a DNA profile can be obtained from both blood colours after several hours and depending on the water environment. Thus, the findings show the potential value of such evidence collected for criminal investigations. Future studies with larger datasets with more replicates and a wider range of conditions will help to understand the potential of such samples for human DNA analysis. Although most researchers have the opinion that washed bloodstains cannot provide sufficient DNA quality for profiling, several studies have shown that washed bloodstains can be used for DNA profiling (Bucka, Hofmann, Rao, & Kamphausen, 2021).

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