

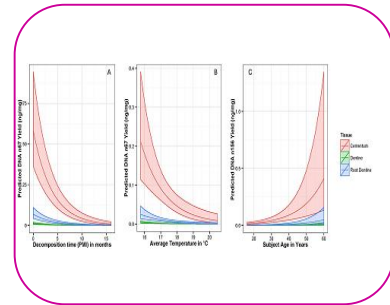


## EFFECT ON DNA PRESENT IN BLOOD STAIN IN DIFFERENT ENVIRONMENTAL CONDITIONS

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### ABSTRACT

Blood is a most imperative organic body fluid that frequently found in the crime scene. Bloodstain play a vital role in crime scene investigation and it is a traceable element and it is a critical legal instrument as conclusive evidence in crime investigation. Investigation of various parts of bloodstains can add clear and essential data can point criminal examination the correct way and help clarify the offence. It is imperative to decide the succession of time of crime amid the responsibility of a brutal crime, which is involved with blood. Investigation of various parts of bloodstains incorporates fitting strategies from Natural sciences, especially techniques in Physical science and furthermore from Mathematics, Biology. The present paper discusses the knowledge implication in the identification of blood in the crime scene from different environment condition and reserves the chance identification of culprit with accuracy. In the wake of discovering that, it is blood by utilizing serological tests, DNA profiles which help to the identification of a person with the appropriate responses about the arrangement of occasions and instruments of production of particular gatherings of bloodstains on the crime scene, the examination is pointed towards the morphological investigation of bloodstains.

**KEYWORDS:** Blood, Crime Scene, DNA etc.

### INTRODUCTION:

Blood is one of the body fluid that plays a vital role in a crime scene investigation. Through the understanding of blood and its other contains analyzed by expert tell us the questions related to the blood like the origin of blood, the height of blood drop from, the angle of a blood drop, origin o blood, wounds on the body and other more crime scene related information can be fetched from the blood. Blood represents in crime scene may tell us the many more information like bloodshed in a crime scene, dragging of the body in a crime scene, serial order of action in the crime scene. When blood comes to contact with the external environment the drying process of blood changes with the conditions of the physical environment. Drying of the blood will depend on the size of the bloodstain, target initiated in the crime scene after the environment. In a similar condition, the bigger size bloodstain will take a longer time to dry than the smaller bloodstain in a similar condition like temperature, humidity, moisture and airflow will change the drying time of bloodstain in the crime scene.

The material that absorbs the bloodstain or permits the soaking will take longer time for drying. The drying bloodstain examines around the edges of stain on ground and central position of stain, where the central position of the bloodstain creates the circular rim and dried which is referred to as skeletonized bloodstain. Another type of skeletonized bloodstain contains the central part of bloodstain dried partially, contact and leaving peripheral rim intact. This phenomenon can determine when the drying process is started and it will help to the reconstruction of the crime scene using the bloodstain.

1. Victims body and clothing spatter with clotted blood indicates the beaten to death, or possibly postmortem infliction injury
2. The clotted bloodstains on road may indicate that impact of the body with more than one vehicle.
3. The blood by coughing and exhalation of the clotted blood of victim indicate that post-injury survival time.

The reproduction of bloodstain clotting, drying time in crime scene and other phenomenon related to the crime scene will be done through the experiment using the fresh blood and similar volume placed, identical surface with same environment condition of the crime scene. The colour change in bloodstain from red to brown and other progress in blood is observed through the different colour change from red to reddish brown and later into dark brown and black. The haemoglobin in the blood is reasons for the colour change in a blood after coming to contact with air.

The environment condition of a crime scene is especially present with a microorganism, bacteria, variable in temperature, moisture, and crime scene bloodstain may open for all another phenomenon for physical environment therefore for understanding the age of blood the preservation of scene is vital. The differently conditioned bloodstain may present in a crime scene, as moisture content may dilute the blood, temperature dries the blood soon, and all other biological particles organism may alter the condition of the blood from the crime scene.

#### ENVIRONMENTAL CONDITIONS:

The study is done based on bloodstain kept for 30 days under observation of different physical environmental condition like.

1. Room temperature
2. Sunlight
3. Soil
4. Humid
5. Extreme heat

#### MATERIAL AND METHODS

##### PREPARATION OF BUFFER SOLUTION

**FGL lysis buffer (50ml):** 155mmol NaCl- 414.54mg 10mmol EDTA- 186.12mg 10mmol NaHCO<sub>3</sub>- 42.005mg

**Proteinase k buffer (50ml):** 30mmol Tris-HCl- 0.18ml or 180 $\mu$ l 30mmol EDTA- .558ml or 558 $\mu$ l 800mmol Gu HCl- 3.82g or 3820mg 5% Tween 20- 2.5ml or 2500 $\mu$ l 0.5% TritonX 100- 0.25ml or 250 $\mu$ l Add fresh proteinase k for a concentration of 100 $\mu$ g/ml prior to digestion. In 50ml: 50 $\times$ 100=5000 $\mu$ g=5mg

**Phosphate buffer saline (50ml):** KCl- 1gm NaCl- 4gm Na<sub>2</sub>HPO<sub>4</sub>- 0.57gm KH<sub>2</sub>PO<sub>4</sub>- 0.1gm Adjust the ph to 7.4 with HCl Distilled water

**EXTRACTION OF DNA** Take 2cm bloodstain into the 2ml tube. Add at least 2ml of 1X PBS to the stain and elute for 2-4 hours at 56 $^{\circ}$ C. Separate the fabric from eluant through a syringe without the needle and discard the fabric pieces. Take the supernatant in a 2ml tube. Add 1ml of ice-cold isopropanol. Mix by inversion. Centrifuge at 14,000rpm for 10min at 4 $^{\circ}$ C. Decant the supernatant. Wash with cold 70% ethidium bromide(300 $\mu$ l) Centrifuge at 10,000rpm for 10mins Decant the supernatant Air dry or incubate in the dry bath at 55 $^{\circ}$ C for 55min Dissolve in nucleus-free water (30 $\mu$ l)

#### GEL PREPARATION:

0.8g agarose. Dissolve in 80ml TBE (1X) buffer. Heat the solution (mix until the solution gets transparent). After cooling add 5 $\mu$ l ethidium bromide. Pour the solution into the glass chamber and put the comb over it for making the wells. Let it dry for some time. Remove the comb after drying and carefully put the glass plate into the chamber. Now fill the chamber with TBE (1X) buffer. Add 5 $\mu$ l loading dye into the blood samples. Load 15 $\mu$ l of the blood sample into the wells from the tube. Load 5 $\mu$ l of 1kb ladder in the well. Electrical current is then turned on (120V) for 20-25mins and the negative charged DNA moves through the positive side of the gel. (Ladder is used to identify the approximate size of a molecule run on a gel during electrophoresis).

## OBSERVATION

- 1. SUNLIGHT:** Sunlight is a presence with the ultraviolet rays (UV) which are active as mutagen agent that causes the mutation in the DNA of Bloodstain. It brings the chemical changes in the shape of DNA it may change the process of correct the DNA code. The wavelength of UV is 200-300 nm (nanometer) the other source of lights emission were absorbed by the DNA like a sponge because most intense part of the sunlight is absorbed by an intense portion of the DNA (250-260 nm) where UV emission complement emission light is 240-280nm.
- 2. ROOM TEMPERATURE:** At room temperature degradation, the rate of DNA is slow. DNA degradation is seen in 8-9 months.
- 3. SOIL:** bloodstain found at crime scene under the soil were result in the fabric as they are very fabricated which are very difficult to analyze. Soil contaminated blood is degraded and this is due to the biological particles, microorganism in the soil, sunlight, moisture in the soil, water content mainly influences the bloodstain in the crime scene. Therefore, with the above-fabricated factors fabric, degraded blood over time is difficult to obtain the DNA and genetic identification from them.
- 4. HUMID:** Presence of water molecules in the atmosphere and in the air that sustains the certain temperature is known as relative humidity. The humidity and water molecule in the air with the physical environment may cause the development of microorganism with reference to the molecule present in the air. Such condition is very helpful to bacteria; microorganisms develop or multiply themselves very fast. The degradation of DNA proteins depends on the presence of water. The microorganism, bacteria and fungi developments in crime scene will rapidly decay the DNA and turn greenish-black.
- 5. EXTREME HEAT:** The hydrogen bonding between nucleotides is disrupted and results in the separation of previously annealed strands. DNA is damaged and becomes darker in colour.

## RESULT

**FRESH BLOOD:** With gel electrophoresis method a positive detection is observed for any volume used. DNA is recovered on all samples and high-quality DNA profiles are obtained. In 0.5ml of a blood sample, 5mg of DNA is extracted.

**SUNLIGHT:** Smearing is observed. A small amount of DNA is also present in wells. 100.12pg of DNA is extracted.

**ROOM TEMPERATURE:** 63.05 ng of DNA is extracted

**SOIL:** Smearing is observed. 46.64ng of DNA is extracted.

**HUMID:** Clarity of other bands and size are reduced. 44.88ng of DNA is extracted.

**EXTREME HEAT:** Clarity of other bands and size are reduced. 70.02pg of DNA is extracted.

## CONCLUSION

Generally, it is seen that DNA can be preserved in dry natural stains for years and can be investigated through different environmental confines. Bloodstains were reserved at different circumstances of relative humidity (RH), soil, sunlight, room temperature, extreme heat and a fresh blood sample was taken for comparison. We extracted a certain amount of DNA from every blood stain sample kept in different conditions but the least amount was obtained from sample undergone through extreme heat i.e. 70.02pg of DNA whereas from the fresh blood 5mg of DNA got extracted. DNA damage also occurs the most in UV light. These findings also demonstrate that we cannot obtain the exact amount of blood present on the fabric from which DNA was extracted as the extraction process was carried out from their supernatant because some blood was stuck with fabric in the debris. Therefore, a comparison can be done because of results are seen on gel electrophoresis and spectrophotometer. The result shows that DNA gets highly degraded in heat, therefore, it is also recommended not to dry any blood sample by using any kind of artificial heat because the maximum amount of DNA damage occurs. Natural air is preferred for drying blood otherwise it becomes difficult to obtain genetic information.

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