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# Identification, Origin and Categorization of Blood from Different Rusted Iron Surfaces with respect to time for Forensic Consideration

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***Abstract:*** *Blood is a highly complex mixture of cells, enzymes, proteins, and inorganic substances. Blood is one of the important biological evidence commonly encountered by the investigators at the scene of violence crime. Blood and blood stains are a very important entity in medico legal practices as factors like source of blood and their stains help in solving the crime of violence, accidental cases. This study was carried out at Sam Higginbottom University of Agriculture, Technology and Sciences, of Allahabad. In the present study, 3 different samples of blood were collected from central pathology lab and 6 different type of Iron samples were also collected from the local shops of Allahabad. The purpose of this study was for the identification of the changes in the characteristics of blood with respects to time. Precipitin test and benzedine test were carried out in the present study. Precipitin test was considered as an optimal test to determine the origin of species from blood. Different results were obtained. Thus it becomes essential to determine the sensitivity of blood stains on any surface. But in most cases of violence the presence of blood on the different rusted iron surfaces leads to false positive or negative tests which in turns results in no option by the experts. So it's very necessary to examine blood which assists the forensic analyst in an enhanced way.*

***Keywords****: Blood, Precipitin test, Sensitivity*

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## I. Introduction

Human blood is a fluid that is also a type of connective tissue. It is composed of blood cells and an aqueous fluid known as plasma. Two major functions of the blood include transporting substances to and from our cells and providing immunity and protection against infectious agents such as bacteria and viruses. Blood is a component of the cardiovascular system. It is circulated through the body via the heart and blood vessels. The blood cells are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood and carbon dioxide from the tissues to the lungs. It transports nutritive substances and metabolites to the tissues and removes waste products to the kidneys and other organs of excretion. It has an essential role in the maintenance of fluid balance. Blood pH is regulated to stay within the narrow range of 7.35 to 7.45, making it slightly basic. Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too basic. A study of the sensitivity and specificity of the phenolphthalein test as an indicator test for blood was reported by **Higaki and Philip (1976)**. The study compared the phenolphthalein and Benzidine tests as presumptive tests. The results in the Benzidine test but not in three-stage phenolphthalein test. Precipitin Test are based on the fact that when animals (commonly rabbits) are injected with human blood, antibodies get formed which reacts with the invading human blood to neutralize its presence. **Uhlenhuth (1901)** took protein from a chicken egg, and injected it into a rabbit. The rabbit's immune system produced antibodies to protect it against the chicken antigens. When Uhlenhuth mixed the rabbit's blood with egg white, the antibodies in the blood reacted with antigens in the egg, marking it separate in a cloudy deposit, which he called **precipitin.** Injecting human cells into the rabbit made the test specific to humans.

## II. Methodology

**Collection of Samples:** Blood samples of different blood groups were collected from Central pathology lab of Motilal Nehru Medical College Allahabad and iron surface were collected from local shop of Allahabad.

**Preparation of Collected Blood Samples:** Blood drops were applied on different surfaces of rusted iron. After drying the blood on different surfaces, it was scraped out and dissolved in normal saline to conduct the examination. The diluted blood samples were examined for their sensitivity towards origin and grouping tests.

**PREPRATION OF AGAR – GEL PLATE**

A glass plate was taken. 300mg of agar power was weighed and placed in a clean test tube and dissolved in 200ml of normal saline by heating the solution on water bath. Dissolved Agarose was carefully poured on glass plate to make 1-2mm thick semisolid layer of gel. Holes were made in gel with metal punch. The size of these holes in 2mm in diameter and the distance between two rows was about 5mm. The agar gel was prepared fresh every time.

**ORIGIN OF SPECIES TEST OF BLOOD:- PRECIPITIN TEST (TUBE METHOD) Procedure –**A portion of stain containing swab was taken in a clear test tube. A small portion of saline was added it and soaked for about two hours to obtain a concentrated extract. The strength of the stain was standardized if persistent foam develops on slight shaking of the saline extract in tube (1/1000 dilution). The diluted blood extract was taken in a precipitin tube. One drop of anti human serum from a pipette was carefully added to the precipitin tube containing the extract by allowing the drop all slowly along the inner wall of tube. After 15-20 min minutes observed for the precipitin band.

**GEL DIFFIUSION METHOD- Procedure**- A portion of stain containing swab was taken in a clean test tube. A small amount of saline was added to it and soaked for about 2 hours to obtain a concentrated extract. The strength of the stain was added to it and soaked for about 2 hours to obtain a concentrated extract. The strength of the stain was standardized if persistent from develops on slight shaking of the saline extract in the tube (1/1000 dilution). Using capillary tubes the swab extract was added in the $1^{st}$ row of the wells and anti human serum was added in the $2^{nd}$ row of the wells. The plates was then kept in moisturized Petridis and left in the refrigerator over a night. Precipitin bands were observed.

## III. Result And Discussion

The study describes how the origin and grouping of species can be accessed from the degraded human blood samples based on their sensitivity towards the precipitation and ABO grouping and how it is affected with the increased time interval between the blood samples and the precipitin and grouping tests. The presence or absence of antigens in a blood sample as well as surface from which the blood samples were collected in determining the sensitivity of blood. Blood from the different iron surfaces was taken and tested for grouping, its sensitivity for the precipitin test with normal saline solution and the results were observed for all the surfaces for a time period of 35 days at different time intervals. Swabs were extracted with the help of normal saline water. The results were observed by rubbing swabs on rusted iron surface which contain blood stain with saline water. Fig. showing the result of Benzidine test which were extracted from normal saline water. All 6 samples has given positive Benzidine test in normal saline water. In case of ring test all 6 samples were give positive results in Gel diffusion method offers considerably better and conclusive result even in low quality of the biological evidence. But the results of best observed for origin is precipitin or ring test considered significant in this study.

**Table.3.1** Results of Benzidine test of Blood samples on Rusted Iron Surface extracted through Normal saline solution

| Serial No. of 1 to 6 Samples | Days | Results of Benzidine Test with samples | Color of Benzidine Test with samples |
|---|---|---|---|
| **1.** | On $1^{st}$ | Positive | Very Dark and clear |
| **2.** | On $7^{th}$ | Positive | Dark color |
| **3.** | On $11^{th}$ | Positive | Dark color |
| **4.** | On $14^{th}$ | Positive | Dark color |
| **5.** | On $18^{th}$ | Positive | Dark color |
| **6.** | On $21^{th}$ | Positive | Dark color |
| **7.** | On $25^{th}$ | Positive | Dark color |
| **8.** | On $28^{th}$ | Positive | Light color |
| **9.** | On $32^{nd}$ | Positive | Light color |
| **10.** | On $45^{th}$ | Negative | Inconclusive |

As shown the table No.3.1. It was found that all the samples were give positive benzidine test.In case of Benzidine test 6 samples collected from rusted iron surface was subjected to Benzidine test in normal saline solution it was observed that initially days of testing the samples gave very dark bluish green color. In initially days all 6 samples give positive reaction. But after 45 days only light color was observed and finally only a inconclusive reaction was observed for the test.

**Table.3.2** Results of Origin determination of Blood samples on Rusted Iron Surface extracted through Normal saline solution

| Case no. of 1-6 samples | Days | Results of Ring Test | Gel Diffusion Test |
|---|---|---|---|
| **1.** | On 1st | very dark agglutination ring | Human |
| **2.** | On 7th | very dark agglutination ring | Human |
| **3.** | On 11th | very dark agglutination ring | Human |
| **4.** | On 14th | light agglutination ring | Human |
| **5.** | On 18th | light agglutination ring | Human |
| **6.** | On 21th | light agglutination ring | Human |
| **7.** | On 25th | light agglutination ring | Human |
| **8.** | On 28th | Very light agglutination ring | Human |
| **9.** | On 32nd | Very light agglutination ring | Human |
| **10.** | On 35th | No agglutination ring found | Human |

As shown the table No.3.2 it was found that all the samples were given positive test in case of ring test and Gel diffusion method. Blood samples collected from different Rusted Iron Surface was subjected to Precipitin test in normal saline solution, it was observed that initially the sample gave very dark precipitin ring in at the base in the initial days of testing for the precipitin test followed by a progressive decrease(dark, light, very light, inconclusive ) in the intensity of the ring and finally only a positive reaction was observed when the sensitivity of the blood towards precipitin test (or anti- human serum) was reduced.

**Grouping**

Blood sample collected from rusted iron surface were subjected to grouping test after diluting it in normal saline water, The extracted blood samples with normal saline water was taken in a test tube then apply to react with Anti-A, Anti-B, and Anti-D.

**Table.3.3** Results of ABO Grouping Identification test of Blood samples on Rusted Iron Surface extracted through Normal saline solution

| Sample no. | Days | ABO Grouping |
|---|---|---|
| **1.** | On 1st | Positive |
| **2.** | On 7th | Positive |
| **3.** | On 10th | Positive |
| **4.** | On 13th | Positive |
| **5.** | On 15th | Inconclusive |
| **6.** | On 18th | Inconclusive |

As shown the table No.3.3 the result for the determination of Grouping of species and it was found that all the samples were given positive test in normal saline solution, it was observed that initially 1 to 7 days the sample give agglutination. But after 10 to 12 days it gives slight agglutination and finally a progressive decrease in the positive reaction was observed for this test. the interesting fact about the study is that blood group with antigens ($B^+$) was least sensitive to the groping test as it was the blood group whose sensitivity was suppressed early as compared to the blood group with antigens ( $A^+$).

## IV. Discussion

Blood samples collected from Rusted Iron Surface was subjected to groping test in normal saline solution, it was observed that initially 1 to 7 days the sample give agglutination. But after 10 to 12 days it gives slight agglutination and finally a progressive decrease in the positive reaction was observed for this test. The interesting fact about the study is that blood group with antigens ($B^+$) was least sensitive to the groping test as it was the blood group whose sensitivity was suppressed early as compared to the blood group with antigens ( $A^+$). The blood samples from suspected rusted iron surfaces were collected from shops. The older bloodstains and denatured stains are sometimes very difficult to extract in sufficient quantity. The extracted samples were examined for the presence of bloodstains, followed by its origin and grouping. The standard methods of preliminary examination of blood were adopted as suggested in previous studies of **Grodsky *et al.* (1951), Culliford and Nickolls (1964) and Cox (1991).** The results was satisfactory in terms of detection of blood and in accordance with **Hass, *et al*.(2009) and Mohite *et al.* (2001).**

## V. Conclusion

During dissertation work 6 samples were identified by preliminary identification of blood using Benzidine test which gave positive test up to 32nd day. For the individualization purpose ABO blood groping was done. After performing the immunological technique (or the precipitin test in this case), it is concluded that human blood is sensitive to precipitin test but only for a specific period of time(up to 32nd day), also the surface

and the presence or absence of antigen in the blood does affect the sensitivity of blood for the anti-human serum. The interesting fact about this study is that the categorization of blood group with antigens ($B^+$) was least sensitive to the precipitin test as it was the blood group whose sensitivity was suppressed early as compared to the blood groups with antigens ($A^+$). It is concluded that the sensitivity of blood is inversely proportional to time, i.e. as the time interval between the blood and the precipitin test increase, the sensitivity for the test decrease,

**Sensitivity of blood ∝ 1/ Time Interval**

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