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Tracing the Clues: Exploring Dried Bloodstain Analysis on Multiple Objects in Varied Environment Conditions

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Abstract:
The primary goal of the study was to detect the presence of blood on different items often encountered at crime scenes. The researchers used a variety of techniques to analyze the blood on these surfaces. Blood samples were drawn from participants and stored in EDTA vials. Six different items were selected for analysis: a living leaf, glass slide, dry leaf, rusting iron, rubber (tyre tube), and stone. The procedure involved applying two to four drops of blood to each surface and subjecting the items to three different temperatures: room temperature, -75 degrees Celsius (in a deep refrigerator), and 150 degrees Celsius (in a hot air oven).The analysis of blood on the surfaces involved three tests: the Benzidine fast blue test, the Takayama test, and the Teichmann test. The Benzidine test produced a strong blue color if blood was present. The Takayama test resulted in salmon pink crystals, and the Teichmann test produced dark brown rhombic crystals – both indicating the presence of hemoglobin.The equipment used for analysis included a compound light microscope with various lenses for magnification. Reagents like Benzidine, glucose, pyridine, NAOH, and KCl/KBr were utilized in the tests. The samples were analyzed at different time intervals, ranging from 3 hours to 75 days after preservation. This allowed researchers to understand the stability of blood on the surfaces over time. To ensure reliable results, the study employed 10 objects for each temperature and time period. This setup facilitated comparative analysis and enhanced the credibility of the examination.
The presence of blood was determined based on the outcomes of the tests and observations made under the microscope. The study's findings could potentially assist in solving crime cases and accidents involving the tested items. Detecting blood on various surfaces under different conditions could contribute valuable information to forensic investigations.

Keywords: Bloodstain analysis, Blood test, Confirmatory test Takayama and Teichmann.

Introduction: Blood is a connective tissue. It serves as the transporting medium for all the substances in the body [1]. Bloodstain pattern analysis [2], which may be connected to the distribution, form, and size of each individual bloodstain as well as the pattern itself, may significantly contribute to the reconstruction of events in the forensic field [3]. Bloodstain pattern analysis has been greatly enhanced

by extensive research on the detection [4], molecular identification, and estimation of deposition time of human traces, but thorough study and knowledge of the physical characteristics of extracorporeal blood droplets are still needed.In addition to volume and surface area, temperature, humidity, air quality, and vapour pressure are the key factors that affect how quickly a liquid dries [5]. Bloodstains can be recognised chemically in a number of ways [6]. The benzidine test is the most frequently used [7]. As the most effective initial test, Lucas suggests the benzidine response. He claims that "in no case has the presence of blood ever been found to produce a negative benzidine reaction." The benzidine test involves creating a solution of benzidine base in glacial acetic acid and dropping it onto wet filter paper that has already been stained by pressing the filter paper up against the stained exhibits [8]. Then hydrogen peroxide is added in a drop. The presence of blood is indicated by a vivid blue-green coloration. In my research, we used the Takayama Test and the Teichmann Test, two confirmation tests that allow us to conclude with confidence that the substance is blood [9]. Hemochromogen crystal assay and immunochromatographic test are additional names for the Takayama testb[10]. The iron atom of the heme group reacts with pyridine to generate a ferroprotoporphyrin ringb[11]. If presence of blood was determined based on the outcomes of the tests and observations made under the microscope [12], it generated a salmon-coloured crystal. The haematin crystal acid test and the haemin test are other names for the Teichmann crystal test. Blood from dried spots is detected using it. In the Teichmann experiment, haemoglobin changed into hemomin crystals, which changed into salt when halogen was present. Rhombic crystals then formed. In the presence of blood, it generated a dark brown coloration and a rhombic shape [same as 9].

Material and Methodology:

Sample Collection In this study, total of 240 ml. Blood was procured from 24 healthy individuals in EDTA vial and kept in 75ºC [same as 6]. All the individuals were informed about the purpose about the study and their written consent was taken.

Material: Six different common objects such as a glass slide, leaveing leaf, stone, dry leaf, rusting iron and rubber (tyre tube) were used as a substrate. These objects are very common at most of the crime scene. The main purpose was that to identify the blood from different surfaces of the objects[13].

Methology: Different samples were collected for the examination of the preserved blood. I had applied 2 - 3 drops of blood on each surfaces. We took these objects in multiples of 10. We had put each object in 3 different environmental temperature. Presumptive tests (Benzedine fast blue test), and confirmatory tests (Takayama and Teichmann test) were done. Room temperature between 28 and 35 degrees Celsius was the first environmental condition. We measured the deep refrigerator's temperature at -75 degrees Celsius. We've measured three temperatures in a hot air oven with a 200° C heat[14]. Keep 10 items together at a single temperature, such as 10 for room temperature, 10 for refrigerator, and 10 for hot air oven, since we perform the same process over a variety of time periods. As we did the first time, we preserved the specimen for three hours, then again for six hours, and finally for twenty-four hours [same as 14].

S. No	Droplet wiped after time
01	03 hrs
02	1 day
03	3 day
04	7 day
05	15 day
06	25 day
07	35 day
08	45 day
09	60 day
10	75 day

Table 1 showed the time duration for the examination of Bloodstain.

Results: The current study focused on the Takayama and Teichmenn blood conformatory tests as well as an analysis of dried bloodstains on various objects recovered at crime scenes in varied environmental conditions.

Time		**3hrs**	**1 day**	**3 day**	**7 day**	**15 day**	**25 day**	**35 day**	**45 day**	**60 day**	**75 day**
Substrate	**Temperature**										
Living Leaf	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Living Leaf	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Living Leaf	200^0 C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Glass Slide	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Glass Slide	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Glass Slide	200^0 C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dry Leaf	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dry Leaf	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dry Leaf	200^0 C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rusting Iron	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rusting Iron	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rusting Iron	200^0	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

	C										
Rubber (Tyre)	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rubber (Tyre)	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rubber (Tyre)	200^0 C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stone	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stone	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stone	200^0 C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

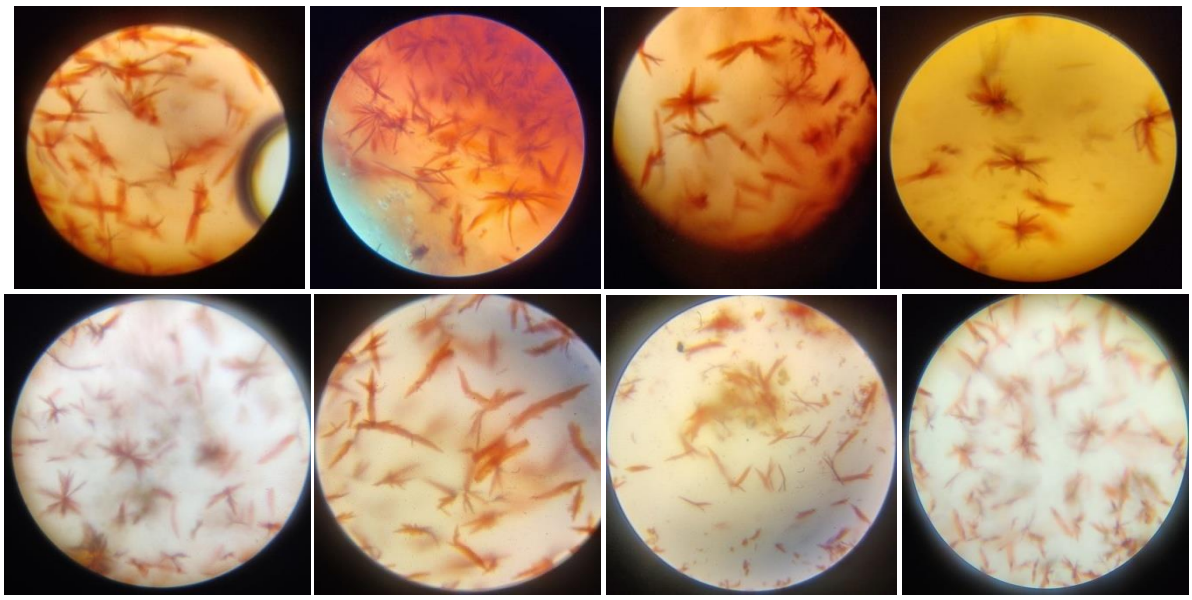
Table 2 Shows the Results of Benzidine Test.

In this study we found that all the blood samples from table 2 it is clear that all the tests of gave positive results over six different surfaces after 3hrs to 75 days. At room Temperature, at -75ºC and 200ºC.

Time		**3hrs**	**1 day**	**3 day**	**7 day**	**15 day**	**25 day**	**35 day**	**45 day**	**60 day**	**75 day**
Substrate	**Temperature**										
Living Leaf	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Living Leaf	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	×	×
Living Leaf	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Glass Slide	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Glass Slide	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	×	×
Glass Slide	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Dry Leaf	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Dry Leaf	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	×	×
Dry Leaf	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Rusting Iron	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Rusting Iron	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	×	×
Rusting Iron	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Rubber	Room	✓	✓	✓	✓	✓	✓	✓	✓	✓	×

(Tyre)	tep.										
Rubber (Tyre)	-75°C	✓	✓	✓	✓	✓	✓	✓	✓	×	×
Rubber (Tyre)	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Stone	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Stone	-75°C	✓	✓	✓	✓	✓	✓	✓	✓	×	×
Stone	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×

Table 3 Shows the results of Takayama Tests and below the table some results images were pasted.

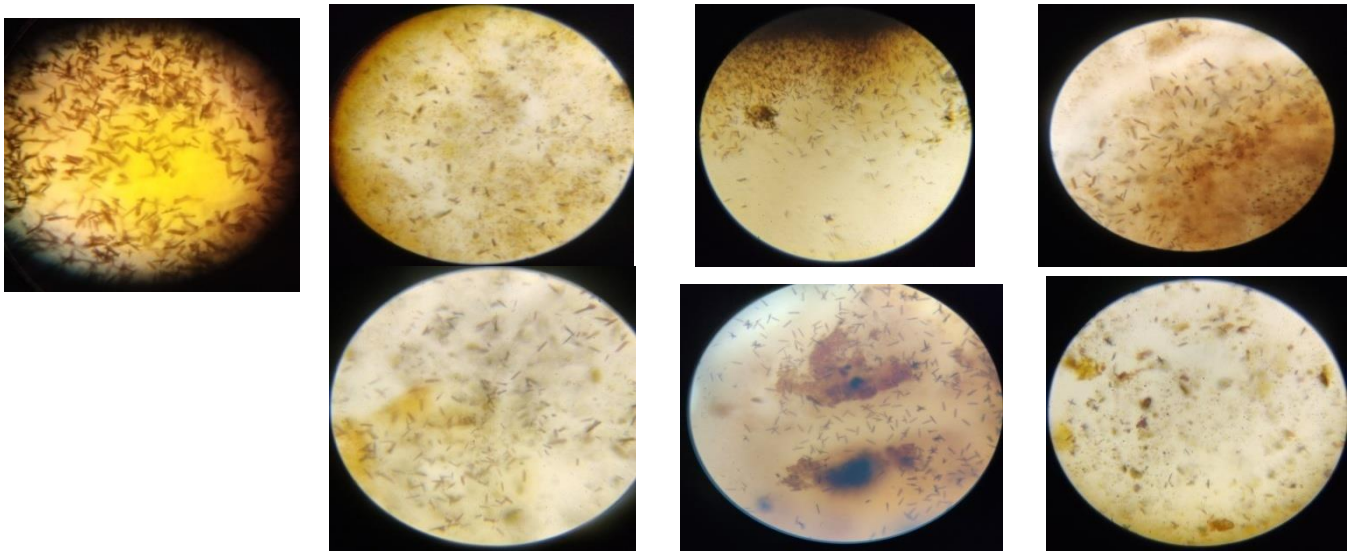


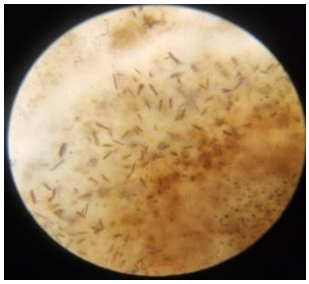
In this study we found that all the blood samples from table 3 it is clear that all the tests of gave positive results over six different surfaces after 3hrs to 45 days. At room Temperature and at -75°C. But in this observation we found that all the tests (takayama) were shows the negative results placed at 200°C after 45 day of the observation time period, after 60 days of the observation time period Takayama test will shows the negative results at -75°C and after 75 days of the observation time period of takayama test will shows the negative results at room temperature.

Time		**3hrs**	**1 day**	**3 day**	**7 day**	**15 day**	**25 day**	**35 day**	**45 day**	**60 day**	**75 day**
Substrate	**Temperature**										
Living Leaf	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Living Leaf	-75°C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Living Leaf	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×

Glass Slide	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Glass Slide	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Glass Slide	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Dry Leaf	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dry Leaf	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dry Leaf	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Rusting Iron	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rusting Iron	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rusting Iron	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Rubber (Tyre)	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rubber (Tyre)	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rubber (Tyre)	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×
Stone	Room tep.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stone	-75ºC	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Stone	200^0 C	✓	✓	✓	✓	✓	✓	✓	×	×	×

Table 4 Shows the results of Teichmann Tests and below the table some results images were pasted.





In this study we found that all the blood samples from table 3 it is clear that all the tests of gave positive results over six different surfaces after 3hrs to 75 days. At room Temperature and at -75^{o}C. But in this observation we found that all the tests (teichmenn) were shows the negative results placed at 200^{o}C after 45 day of the observation time period.

Summary:

The study's primary objective was to detect blood on items often found at crime scenes using diverse techniques. Blood samples from participants were stored, and six surfaces including a living leaf, glass slide, dry leaf, rusting iron, rubber, and stone were tested. Blood drops were applied to each surface and subjected to room temperature, -75°C (deep fridge), and 150°C (hot air oven). Three tests – Benzidine for blue color, Takayama for salmon pink crystals, and Teichmann for brown rhombic crystals – were used to identify blood presence. Analysis employed a compound light microscope and reagents like Benzidine. Over time (3 hours to 75 days) and varying temperatures, stability was studied using 10 objects for each setting. Results were determined via tests and microscopy. This research holds potential for solving crime cases by detecting blood on surfaces, thus aiding forensic investigations. In summary, the study investigated blood detection on common crime scene items, utilizing diverse tests, temperatures, and timeframes to understand stability and practical applicability.

Discussion:

It is found that the findings of the two confirmatory tests are consistently positive, with the exception of a 45-day window during which the Takayama and Teichmann tests also produced negative results when conducted at 200°C in a hot air oven. Through this experiment, we acquired proof that dried bloodstains in Takayama confirmatory tests change negatively after 60 days of being left at room temperature. We observed that the blood samples in the hot air oven were destroyed due to the temperature effect, yielding a negative confirmatory result.

According to **Gupta Shivangi (2017)** Benzidine and TMB in acetate buffer solution have similar sensitivity for blood detection. TMB in acetate buffer solution works better for liquid blood; however, solution of TMB in glacial acetic acid works better for stains. LMG solution with zinc has higher sensitivity as compared to a solution without zinc. Luminol is the most sensitive presumptive test for detecting blood on washed and diluted bloodstains, and sensitivity decreases with an increase in wash cycles. Among confirmatory tests, the Takayama test is more promising than Teichmann. Similarly in the current study we have taken benzidine test for room temperature, at -75^{o}C and at 150^{o}C the results showed the positive result of the Benzidine Test.

Conclusion:

This study focused on detecting and analyzing blood on different surfaces commonly encountered at crime scenes. By applying various tests and observing the samples over different time periods and temperatures, researchers aimed to provide insights into the stability and detect ability of blood on these surfaces. Environmental elements including temperature, humidity, and weather might harm the crime scene's evidence. There are many other time periods, including 75 days, 60 days, 45 days, 35 days, 25 days, 15 days, 7 days, 3 days, 1 day, and 3 hours. It has been found that the findings of the two confirmatory tests are consistently positive, with the exception of the 45-day window during which they are negative at 200°C in a hot air oven and the Takayama and Teichmann test results are likewise negative. Through this experiment, we acquired proof that dried bloodstains in Takayama confirmatory tests change negatively after 60 days of being stored at room temperature. A negative confirmatory result was obtained because the blood samples in the hot air oven were destroyed due to the temperature effect. This study discovered that blood and chloroplast interacted, leading microscopic examination to display red color on the lamina of leaves as well as on the midrib and veins of leaves.

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