IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

ESTIMATION OF BLOODSTAIN AGING ON DIFFERENT SURFACES AND DIFFERENT FABRICS

Author: Harshitha K Reg No.: 23MSFS132

Guide: Dr. Surender Kumar Pal, Dr. Ajay Singh Rana, Suhas S and Prof. Joemon K J Designation: Student

Department of Forensic Science Garden City University

ABSTRACT

The age of a blood stain can be used to estimate or to identify the time, when the crime was committed. Blood represents about 8% of body's total weight and has an average volume of 5 liters in women and 5.5 liters in men. Blood travels from the heart to tissues and cells of the body through the distributed blood vessels. RBC's are fully packed with hemoglobin (Hb) which is an iron containing molecule that can bind with O2 loosely and reversibly. As the blood ages deoxyhemoglobin (HbO2) is converted into methemoglobin (MetHb), which changes the colour of the blood, red to brown. This research is to estimate the age of an bloodstain on different surfaces like, glass, tile piece, stones and wood and different fabrics like cotton, silk, velvet and woolen. The bloodstain samples were observed from Day1 to Day40.In this research the environmental factors like temperature and humidity plays an important role. To analyse the age of an bloodstain, we are using visual inspection , Alternative light sources like UV light and Infrared light,and chemical testings like , O- Tolidine and phenolphthalein test. In this research the change of an blood colour were observed on the different surfaces and fabrics and to identify which chemical test shows better results on the aged bloodstains.

Keywords: Age of an Bloodstain, Different surfaces and fabrics, Alternate Light Sources, Chemical Testing and Colour change of Bloodstain

CHAPTER-1

INTRODUCTION:

The blood is a body fluid in the circulatory system of humans and other vertebrates that delivers necessary substances such as nutrients and oxygen to the cells, and transports metabolic waste products away from those same cells. Blood is composed of blood cells suspended in blood plasma. Blood contains plasma, Red blood cells (RBC's) or Erythrocytes, White blood cells (WBC's) or Leukocytes and Platelets. The plasma contains, water, electrolytes, nutrients, hormones and proteins. RBC's carry oxygen from the lungs to body tissues and transport carbon dioxide from the body. WBC's helps to fight infection and disease, it has three types of WBC's, Lymphocytes, Monocytes and Granulocytes and the Platelets helps with Clotting.

Blood is one of the most commonly encountered types of physical evidence at crime scenes involving violent crimes. Its presence can provide not only biological information about the victim or perpetrator, but also contextual clues about the crime itself. Due to its high visibility and biological richness, blood often becomes a focal point for forensic analysis. The study of bloodstains has evolved into a sophisticated brsnch of forensic science known as Bloodstain Pattern Analysis (BPA),which includes the assessment of droplet patterns, directionality, impact angles, and distribution. More recently,an important advancement within BPA is the estimation of the age of a bloodstain, which can be critical for establishing the timeline of a crime. Determining how long a bloodstain has been present at a scene helps forensic investigators differentiate between primary and secondary crime scenes, verify alibis, and exclude or include suspects. Despite its significance, bloodstain aging remains one of the most complex and underdeveloped areas of forensic science.

Blood is a complex fluid composed of cellular components (erythrocytes, leukocytes, platelets) suspended in plasma. The red blood cells carry hemoglobin (Hb), a metalloprotein with four subunits containing iron atoms capable of reversibly binding with oxygen. This interaction is crucial for oxygen transport but also contributes to color changes in bloodstains as hemoglobin degrades over time. In a freshly deposited bloodstain, Oxyhemoglobin (HbO2) dominates, giving blood its bright red appearance. Upon exposure to air and environmental conditions, this molecule oxidizes to form Methemoglobin(MetHb), which appears brownish in color. Eventually, MetHb further degrades into hemichromes, producing a dark brown or nearly black stain. These biochemical transitions occur progressively and afre highly sensitive to environmental factors such as temperature, humidity, light exposure, and the nature of the surface. While the degradation sequence follows a predictable pattern, the rate and visual indicators can differ significantly depending on the external conditions and substrate interactions.

In Forensic science, bloodstains are essential evidence used to unravel the sequence of events at a crime scene. Among the multiple aspects of bloodstain analysis; such as pattern interpretation DNA extraction, and source determination; bloodstain aging holds unique importance. Despite its importance, this domain remains a complex and underexplored area due to the intricate physical and chemical changes blood undergoes over time. Environmental factors like temperature, humidity, light exposure, and surface properties significantly influence these changes, posing challenges for standardization. Forensic experts frequently encounter variability n bloodstain visibility, texture, and chemical responses across different surfaces and fabrics, further complicating accurate aging.

One of the most important yet challenging aspects of bloodstain aging is the influence of environmental variables. The two primary factors considered in this study are temperature and humidity. Temperature affects the rate of oxidation and evaporation. Warmer temperatures tend to accelerate the drying and degradation of hemoglobin, leading to quicker transitions in color and chemical composition. However, excessively high temperatures may also cause desiccation, which can mask underlying changes. And humidity influences the moisture content of the blood and the surface-substrate interaction. High humidity can slow down drying, preserve the liquid state longer, and affect the hemoglobin degradation timeline. Other environmental elements such as exposure to sunlight, artificial lighting, airflow and microbial activity can also interfere with aging processes. These interactions highlight the need for controlled and systematic experimentation to determine bloodstain aging in realostic yet measurable conditions.

The substrate upon which blood is deposited plays a major role in how it dries, absorbs, and degrades. Surfaces (Glass, Tile Piece, Stone & Wood) can broadly be divided into two categories:

- Non-Porous Surfaces like Glass and Tile piece do not absorb liquid, causing blood to pool and remain exposed to air. This often leads to faster oxidation and hemoglobin degradation.
- Porous Surfaces such as Wood & stone absorb blood partially or completely, which can slow oxidation but also complicate visual detection due to stain spreading, diffusion, or seepage.

Fabrics, in particular, add another layer of complexity. Materials such as cotton, silk, velvet and wool differ in their weave, absorbency and chemical composition:

- Cotton absorbs quickly, leading to wide diffusion and potential color changes within fibers.
- Silk has a smooth surface but absorbs well, potentially showing clear edges and color transitions.
- Velvet traps blood within its pile, possibly preserving moisture and slowing aging.
- Wool is fibrous and may promote microbial growth, which can alter chemical characteristics.

Thus, each surface-fabric combination presents a unique environment for blood degradation, requiring targeted observation and analysis.

The most immediate and accessible method of analyzing bloodstain aging is through visual observation. Color changes in blood provide important temporal markers. Initially bright red, blood begins to darken within a few hours. Over time, brown and eventually black hues develop. However, these changes are qualitative and subjective, necessitating support from more objective methods. In this study, two chemical tests are used to support visual analysis:

1. **O-Tolidine Test:** This test is based on the oxidation of the reagent in the presence of hemoglobin and hydrogen peroxide, producing a blue-green color. It is highly sensitive but may react with other oxidizing agents.

2. **Phenolphthalein Test (Kastle-Meyer Test):** This test uses phenolphthalein, which turns pink in the presence of hemoglobin and hydrogen peroxide. While specific and quick, its efficacy reduces as hemoglobin breaks down over time.

Both tests rely on the peroxidase-like activity of hemoglobin. As the protein degrades, its ability to catalyze the reaction diminishes. By observing how the intensity and reaction time of these tests change over a 40-day period, the study seeks to determine which test is more reliable on aged bloodstains and how results differ by substrate.

Forensic analysis increasingly employs Alternate Light Sources (ALS) such as Ultraviolet (UV) and Infrared (IR) lighting to enhance the visibility of stains and biological residues. These methods are non-destructive, rapid, and field-deployable, offering an excellent complement to visual and chemical tests.

- **UV Light** can cause biological stains to fluoresce or appear more distinct due to the interaction with specific proteins or hemoglobin derivatives.

- **IR Imaging** helps visualize blood on dark fabrics or complex backgrounds by detecting absorbance differences.

As blood ages, its fluorescence and absorbance properties may change, making ALS an indirect indicator of age. This study evaluates how bloodstains on various materials react to UV and IR light over time, and whether such responses provide reliable markers for aging.

Bloodstain aging is a nuances, multi-factorial process influenced by chemical, environmental, and substrate-soecific factors. A comprehensive understanding requires not just theoretical knowledge, but systematic obseravtion and empirical validation. This research integrates three practical approaches; Visual analysis, Alternate light sourcw techniques and Chemical testing to estimate the aging of bloodstains across a variety of real-world materials.

By correlating the degradation characteristics of blood with specific surfaces and conditions, this work contributes to the scientific foundation and parctical tools necessary for effective crime scene analysis. It hopes to bridge the gap between laboratory research and field application, ultimately enhancing the accuracy and reliability of forensic timelines.

Aim and Objectives:

Aim: This study aims to develop a comparative understanding of bloodstain aging on different surfaces and fabrics through a combination of Visual observation, Alternate light source (ALS) application, and chemical testing to identify which shows the better results on aged blood stains.

Objectives:

1. To analyze the visual color changes in bloodstains from Day 1 to Day 40 on different materials.
2. To evaluate the reaction patterns of aged stains using O-Tolidine and Phenolphthalein Tests.
3. To assess the effectiveness of UV and IR Light in visualizing bloodstains over time.
4. To identify which surfaces(glass, tile piece, stone & wood) and fabrics(cotton, silk, velvet & wool) preserve blood characteristics longer.

CHAPTER-2

Review Of Literature:

1. The Estimation of Age of Bloodstains by HPLC Analysis; Jan Andrasko (1997)

This study presents a novel High-Performance Liquid Chromatography (HPLC) method for estimating the age of bloodstains on clothing. Researchers identified multiple aging processes, including a decomposition peak labeled "X," which had been reported earlier. These

processes allowed for independent age estimation, provided the storage temperature was known. Notably, the ratios of peaks generated by the aging process were found to remain consistent across a temperature range of 0°C to 37°C. This approach holds promise for forensic applications but requires controlled conditions to yield accurate results.

2. Age estimation of Bloodstains by Hemoglobin derivative determination using reflectance spectroscopy; Rolf H. Bremmer and et.al. (2011)

This research paper investigates the age estimation of bloodstains using reflectance spectroscopy. It demonstrates that the fractions of hemoglobin derivatives—oxyhemoglobin, methemoglobin, and hemichrome—change predictably over time, enabling the determination of bloodstain age under controlled conditions. By applying light transport theory, the study correlates spectroscopic changes to chemical composition transitions in bloodstains. The findings highlight the potential of diffuse reflectance spectroscopy as a reliable forensic tool for estimating bloodstain age, emphasizing the need for further evaluation to adapt this method for practical crime scene applications.

3. Drying Properties of Bloodstains on common indoor surfaces; Frank Ramsthaler and et.al. (2012)

This research paper investigates the drying properties of small blood droplets on common indoor surfaces under varying environmental conditions. It highlights how ambient temperature significantly influences the drying process, with higher temperatures accelerating drying and lower temperatures prolonging it. The study also examines the impact of surface materials, noting differences between wood and linoleum, and finds that anticoagulant treatment does not affect drying times. These findings underscore the importance of documenting ambient conditions at crime scenes to estimate the time elapsed since bloodstain deposition, aiding forensic investigations.

4. Identification and Age Estimation Of Blood Stains On Colored Backgrounds By Near Infrared Spectroscopy; Gerda Edelman and et.al.(2012)

This research paper investigates the application of near-infrared (NIR) reflectance spectroscopy for non-destructive identification and age estimation of bloodstains on colored backgrounds, addressing challenges faced with traditional visible spectroscopy on dark substrates. NIR spectroscopy achieved 100% sensitivity and specificity in distinguishing blood from other substances and successfully estimated the age of bloodstains up to one month old, with a root mean squared error of prediction of 8.9%. The study highlights the potential of NIR spectroscopy as a practical forensic tool for providing temporal insights into crime scenes while accommodating diverse background colors, advancing forensic casework methods significantly.

5. Study on the relations between Bloodstain age on the Cotton fabric with color and color difference; Baozhong Sun and et.al. (December 2015)

This study examines the relationship between the age of bloodstains on cotton fabric and their color differences, using a color chromatic aberration method. The research highlights how changes in bloodstain color over time can provide a new perspective for determining the postmortem interval (PMI) in forensic investigations. By focusing on fabric-specific characteristics, this method offers a practical approach to quickly estimate bloodstain age, aiding in the rapid resolution of criminal cases.

6. Alternative method for Determining the original drop volume of Bloodstains on Knit fabrics; Jingyao Li and et.al. (2016)

This research paper presents a novel, non-destructive method for determining the original drop volume of bloodstains on cotton single jersey knit fabrics, commonly analyzed in forensic investigations. By using an artificial blood substitute to replicate stain characteristics, the study calculates the original drop diameter with high accuracy, minimizing errors in Reynolds and Weber numbers. This approach enhances the reliability of bloodstain pattern analysis, providing valuable insights into the forces and dynamics involved in bloodshed events, and supporting forensic interpretations at crime scenes.

7. Practical Implementation of Bloodstain age estimation using Spectroscopy; Gerda J. Edelman and et.al. (2016)

This research paper explores an innovative approach for estimating the age of bloodstains at crime scenes using spectroscopy. It introduces a refined two-layered light-transport model, enabling accurate determination of the relative concentrations of OxyHemoglobin, MetHemoglobin, and Hemichrome in bloodstains across various colored surfaces. The study employs statistical methods to provide confidence intervals for calculated bloodstain ages and demonstrates practical applicability in real-world forensic investigations, including a shooting incident case study. By offering insights into the sequence and timing of blood shedding events, this method aids in reconstructing crime timelines, validating witness accounts, and prioritizing forensic sample analyses, representing a significant advancement in forensic science practices.

8. Predicting the time of the Crime:Bloodstain aging estimation for up to two years;Kyle C.Doty and et.al.(2017)

This study explores the use of Raman spectroscopy to estimate the age of bloodstains for up to two years, aiding forensic investigations. Researchers analyzed spectral changes in bloodstains, correlating them with natural biochemical processes. They achieved a 70% accuracy rate in predicting the time since deposition (TSD) using regression models like partial least squares regression (PLSR) and principal component regression (PCR). The findings demonstrate the potential of nondestructive techniques for identifying bloodstains and estimating their age, offering valuable insights for crime scene analysis.

9. Investigating the color of the Bloodstains on Archaeological cloths: The case of the Shroud of Turin; A. Di Lascio and et.al. (2018)

This research paper investigates the unusual reddish color of bloodstains on archaeological textiles, specifically focusing on the Shroud of Turin. The authors test two hypotheses: the presence of carboxyhemoglobin in the blood and the influence of ultraviolet light on high- bilirubin blood over time. Using spectral reflectance analyses and ultraviolet light experiments on stained linen, the study provides insights into the chemical and physical properties responsible for the distinctive blood coloration. The findings contribute to the understanding of stained textiles, offering implications for studies of historical and archaeological artifacts.

10. Highly sensitive & accurate estimation of Bloodstain age using Smartphone; Wooseok Choi and et.al. (2019)

This research paper introduces a smartphone-based pattern recognition system for accurately estimating the age of bloodstains, enhancing sensitivity in early time zones compared to previous methods. It employs three detection techniques—blood pool analysis, crack ratio analysis, and colorimetric analysis—during the drying process of bloodstains. By analyzing images of bloodstains on various materials like glass, wood, paper, and fabric, the system successfully determines bloodstain age at intervals of 9, 18, and 48 hours. The study highlights the potential of this innovative approach to provide real-time forensic insights, aiding crime scene investigations with improved accuracy and efficiency.

11. Determining the Age of Bloodstains at Crime Scenes using ATR FT-IR Spectroscopy and Chemometrics; Will Wetzel (Sep 16, 2024)

This study explores using Attenuated Total Reflectance Fourier Transform Infrared (ATR FT-IR) spectroscopy combined with chemometrics to estimate the age of bloodstains at crime scenes. By analyzing 960 bloodstains on various surfaces (fabric, paper, and glass) under different conditions over 212 days, researchers developed predictive models using Partial Least Squares Regression (PLSR). These models demonstrated high accuracy, particularly on non-rigid surfaces like fabric and paper. A global model for non-rigid surfaces proved versatile for real-world forensic applications. This research highlights ATR FT-IR spectroscopy as a promising tool for accurately determining bloodstain age, enhancing forensic investigations.

12. The Persistence of Bloodstains on buried fabrics & robustness of the Leucomalachite green test in detecting aged Bloodstains on buried fabrics; Cenya Russell and et.al. (2025)

This research paper investigates the persistence of bloodstains on buried fabrics and evaluates the robustness of the Leucomalachite Green (LMG) test in detecting aged bloodstains over a nine-week burial period. It examines cotton, polyester, and wool fabrics, noting significant differences in bloodstain degradation by fabric type, with wool showing the highest persistence. The study demonstrates that burial in soil accelerates the degradation of bloodstains, impacting detectability and evidence preservation. Wool proved the most resilient, retaining stains longer than cotton and polyester. The paper also highlights limitations of the LMG test under these conditions and discusses its implications for forensic practices, including evidence prioritization and the interpretation of results in violent crime investigations. The findings emphasize the importance of timely recovery and careful analysis of buried forensic evidence.

CHAPTER-3

Materials and Methods :

Materials:

1.Blood: The fresh blood was collected in an 3 EDTA vials of about 5ml sample in an each vial.

2.Different Surfaces:

Glass Surface, Tile Piece Surface, Stone Surface and Wood Surface.

1. Glass Surface

- **Composition:** Glass is mainly composed of Silica (SiO_2) with additives like Sodium oxide (Na_2O) and Calcium oxide (CaO). Other Oxides such as Aluminium, Magnesium, or Boron may be present depending on the glass type.The surface may also contain Hydroxyl groups (-OH) and Monile Alkali ions (Na^+, K^+).

2. **Tile Piece Surface:**

Composition: Tile surfaces are engineered from a combination of natural minerals and industrial additives, tailored to achieve desired mechanical, thermal, and aesthetic properties. The core of most ceramic tiles consists of:

Clay minerals (primarily kaolinite) – provide plasticity and structural base

Quartz (SiO_2) – enhances mechanical strength and thermal resistance

Feldspar – acts as a flux, lowering the melting point and forming a glassy phase during firing

Other additives – such as alumina, calcium carbonate, or talc are added to refine texture, control shrinkage, and improve performance during sintering

For glazed tiles, the surface is coated with a thin layer of glass-forming oxides (mainly silica) mixed with colorants (e.g., cobalt, iron, copper oxides) and opacifiers like zircon. This glaze is applied before firing and vitrifies into a hard, impermeable surface.

3. Wood Surface:

Composition: Wood is a naturally derived organic composite material, consisting mainly of plant cell walls organized into a strong yet flexible structure. Its composition includes:

Cellulose (40–50%): A polysaccharide that forms the structural framework of cell walls, providing tensile strength and flexibility.

Hemicellulose (20–30%): A matrix polysaccharide that binds with cellulose fibers and contributes to the mechanical properties and moisture interactions of the wood.

Lignin (20–30%): An amorphous polymer that binds cellulose and hemicellulose together, lending rigidity and decay resistance.

Extractives (1–10%): These are species-specific organic compounds like resins, tannins, oils, and pigments that influence color, scent, and durability.

Wood is classified into:

Hardwood (e.g., oak, maple, teak) – from angiosperms, generally denser and stronger

Softwood (e.g., pine, cedar) – from gymnosperms, lighter and less dense.

4. Stone Surface:

Compostion: Stone surfaces are composed of naturally occurring geological materials, each formed through complex physical and chemical processes over millions of years. The exact composition depends on the stone type:

Granite: Composed primarily of quartz (SiO_2), feldspar ($KAlSi_3O_8$ or $NaAlSi_3O_8$), and mica. It is an igneous rock known for its high hardness and durability.

Marble and Limestone: Both are composed mainly of calcite ($CaCO_3$). Marble is a metamorphosed form of limestone, offering a crystalline appearance and smoother finish.

Sandstone: Predominantly composed of quartz grains bound together by natural cementing agents like silica, calcite, or iron oxides.

Slate: A fine-grained metamorphic rock formed from shale, containing mica, chlorite, and quartz, often in layered form.

These stones may contain trace elements such as iron, magnesium, or aluminum, which influence coloration and weathering behavior.

3.Different Fabrics:

Cotton Fabric, Silk Fabric, Velvet Fabric and Woolen Fabric.

1.Cotton Fabric:

Composition: Cotton is a natural cellulosic fiber obtained from the seed hairs of the Gossypium plant. It is primarily composed of:

Cellulose (~90–95%): A linear polysaccharide made of β-D-glucose units, forming the backbone of the cotton fiber structure. Cellulose is responsible for the fiber's strength, flexibility, and ability to absorb moisture.

Minor constituents: Waxes, proteins, and pectins (~5–10%): Naturally occurring compounds on the fiber surface that influence surface friction, dye uptake, and feel.

Mineral content: Trace elements like potassium, calcium, or magnesium may be present depending on soil and processing.

During textile manufacturing, cotton fibers are spun into yarns and woven or knitted into fabric. Finishing treatments such as bleaching, mercerization, dyeing, or coating alter the final surface behavior.

2.Silk Fabric:

Composition: Silk is a natural protein fiber produced by the silkworm (Bombyx mori), primarily during cocoon formation. Its chemical composition includes:

Fibroin (~70–80%): The structural core protein made of repeated sequences of glycine, alanine, and serine amino acids, forming β-sheet crystalline structures that give silk its strength and luster.

Sericin (~20–30%): A gummy protein that coats fibroin filaments, helping to hold them together during cocoon formation. It is typically removed during processing (degumming) to improve softness and sheen.

Minor constituents: Traces of waxes, pigments, and mineral salts may be present depending on the silk type and processing method.

3.Velvet Fabric:

Composition: Velvet is a woven fabric characterized by a dense pile of evenly cut fibers that give it a soft, plush surface. Unlike silk or cotton, velvet is defined more by its structure than a single fiber type. Common fiber compositions include:

Silk Velvet: Traditionally made from pure silk, offering a luxurious feel and deep luster.

Cotton Velvet: Made from cotton fibers, resulting in a denser, more matte finish.

Synthetic Velvet: Made from polyester, rayon, nylon, or blends, which mimic the softness and sheen of natural velvet with enhanced durability and affordability.

The structure involves two warp threads: one forms the base fabric, and the other forms the pile, which is cut during weaving to create a uniform, upright texture.

4. Woolen Fabric:

Composition: Woolen fabric is made from short-staple wool fibers derived primarily from the fleece of sheep, although other animal fibers (e.g., alpaca, goat, or camel) may be used. Wool is a natural protein fiber, composed mainly of:

Keratin (~95%): A fibrous protein rich in cysteine, forming disulfide bonds that give wool its strength, elasticity, and resilience. Wool fibers are amorphous with some crystalline regions, enabling moisture absorption and thermal regulation.

Lipids and Epicuticle Layer (~1–2%): Natural waxy coatings help repel water and provide slight resistance to dirt and microbial attack.Woolen fabrics differ from worsted wool, as they are made from carded rather than combed fibers, resulting in a fuzzier, bulkier texture with a more insulating surface.

4.Pipette: With the help of the pipette stain the blood on the each surfaces and the fabrics.

5.Marker: Note down the days on the top of the surfaces and an fabrics.

Sample Collection:

Blood:

For blood sample 3 EDTA vials were collected from different individuals, approximately 5ml sample in each vial.

Sample Preparation:

For the preparation of samples, the first step involved selecting four different surfaces and four different fabrics,the four different surfaces are Glass,Tile Piece,Stone and Wood and the different fabrics like , Cotton,Silk,Velvet and Wool fabric. Each fabric was meticulously cut into small, uniform cube-shaped pieces to ensure consistency in the experimental process. A total of four pieces were prepared from each type of fabric and the surfaces were also four in number for consistency of experiment. This systematic approach was adopted to facilitate a comprehensive and controlled study of the effects of blood on various surfaces and fabrics with the passage of time.

- After preparing the samples, with the help of a pipette carefully added 100 microliters of blood on each surface and fabric.
- After adding blood samples on each surface and fabric , they were left for drying at room temperature without any disturbances.
- After 5 days one sample from each surface and fabric were examined under different parameters (Visual Observations, ALS and Chemical Testing).

- Same process were repeated till 40 days with a gap of 5 days without any disturbances at room temperature.

Sample Analysis:

For Surfaces :

Analysis of bloodstain aging was done on the bass of the following methods:

- **Visual Observation:** The samples were observed visually under the normal daylight.
- **Alternate Light Source:** This method was used to detect bloodstains on surfaces (glass, tile piece, stone & wood) by enhancing their visibility under specific light wavelengths for UV Light 420LP & for IR Light 780LP .
- **Chemical Testing:** This chemical testing were performed on each and every surfaces on 40th day. This tests were performed to identify the detection of aged bloodstains.

For Fabrics:

For the analysis of bloodstain aging, there are three methods:

- **Visual Observation:** The samples were observed visually under the normal daylight.
- **Alternate Light Source:** This method was used to detect bloodstains on fabrics (cotton,silk, velvet & woolen) by enhancing their visibility under specific light wavelengths for UV Light 420LP & for IR Light 780LP
- **Chemical Testing:** This chemical testing were performed on each and every fabric on 40th day. This tests were performed to identify the detection of aged bloodstains on different fabric.

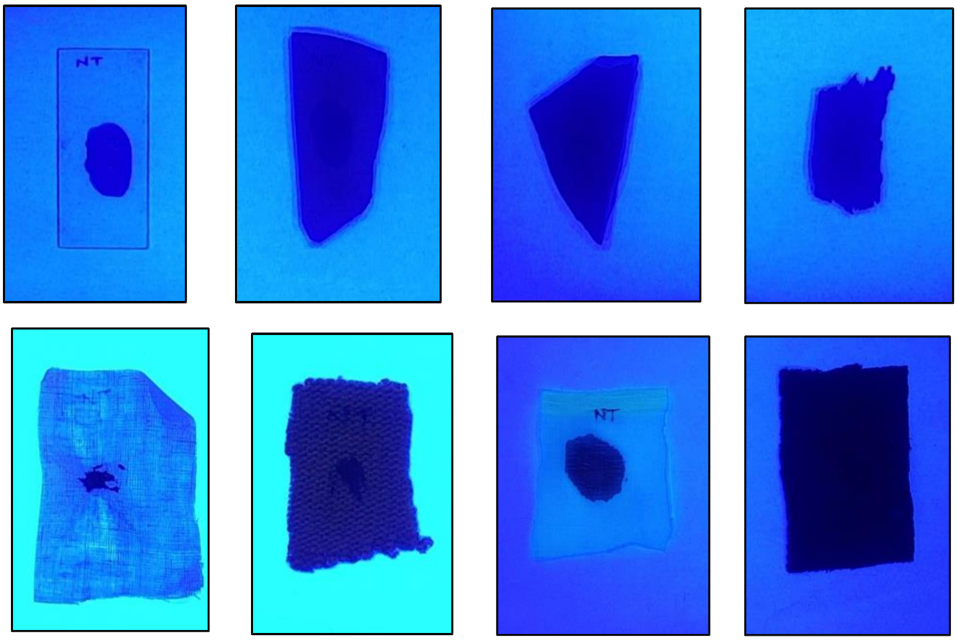
CHAPTER-4

Observation

Day-5/ Neat Samples White Light

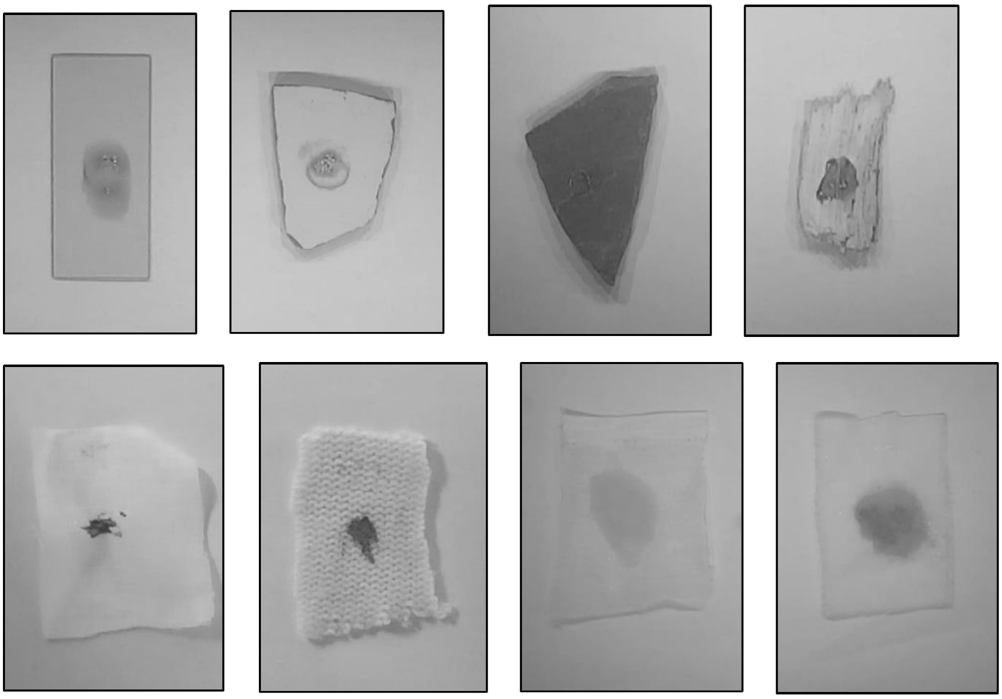
- Reddish stains were obesrved on surfaces; glass, tile piece, stone & wood and fabrics; silk, cotton and woolen fabrics but not on the velvet fabric.

UV Light



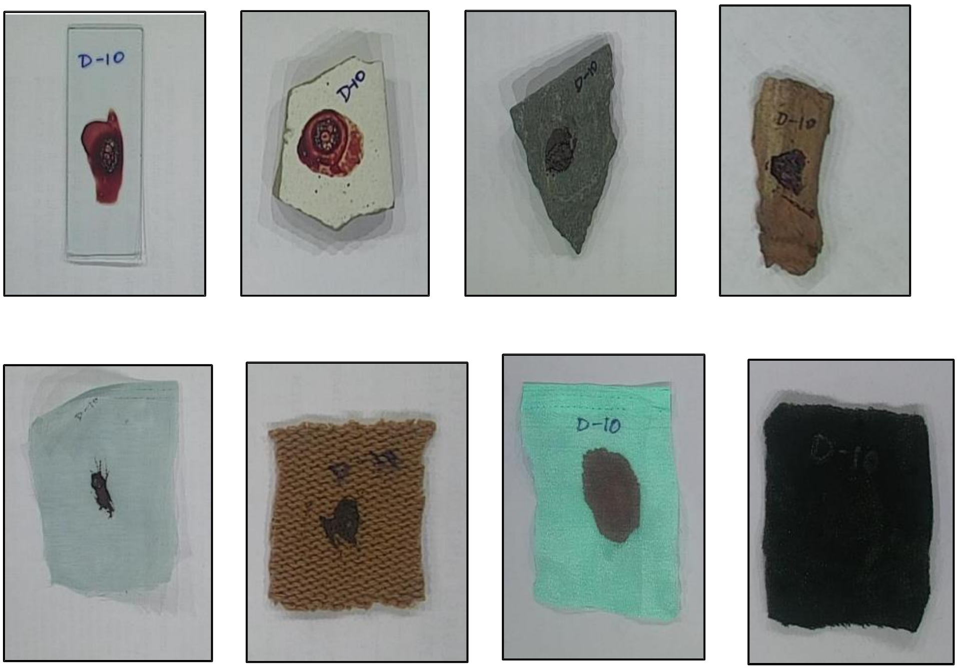
- The stains were observed on glass and tile surface but not on the wood and stone surfaces.
- The stains were observed on cotton, woolen and silk but not on the velvet fabric.

IR Light



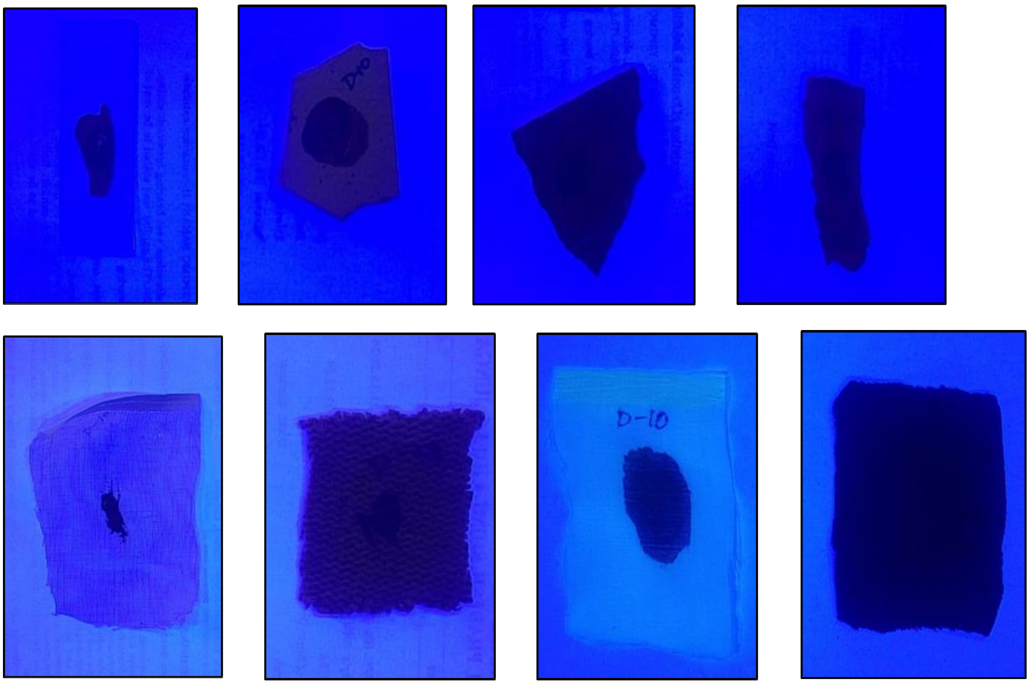
- The stains were observed on surfaces;glass, tile piece, stone & wood and fabrics; cotton,woolen,silk & velvet, but on the stone surface only the outer ring of the bloodstain was observed.

Day-10 White Light



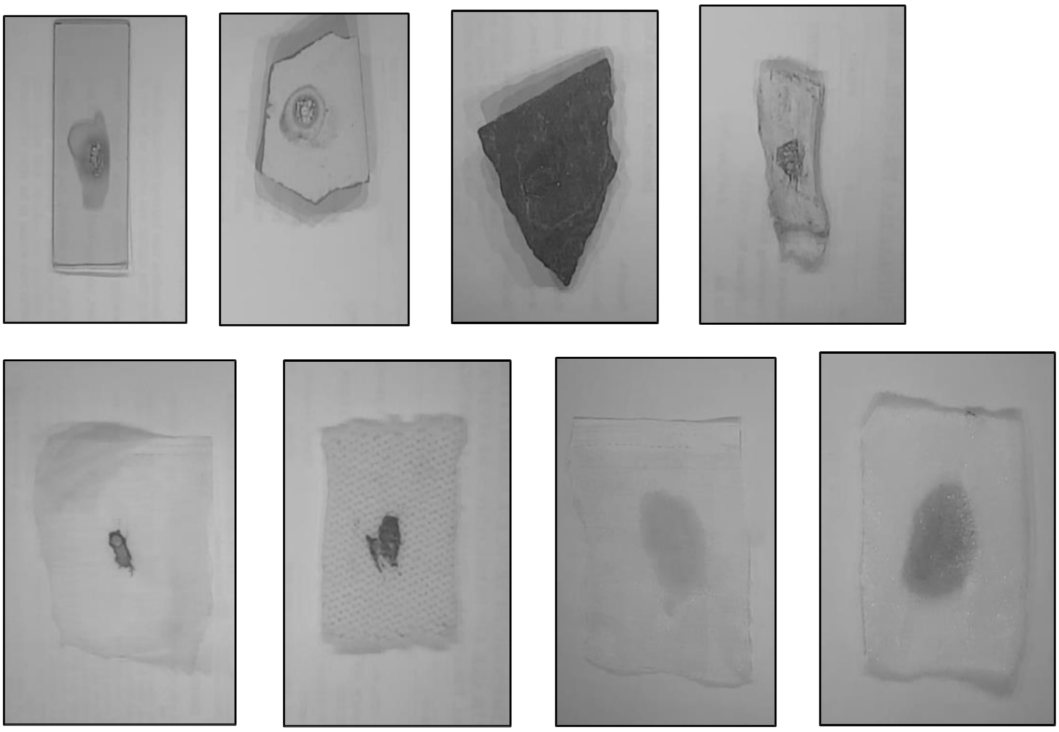
- The reddish stains were observed on surfaces;glass, tile piece, stone & wood and fabrics; cotton, woolen & silk but except on the velvet . Few cracks and scrapings on the glass surface were observed.

UV Light



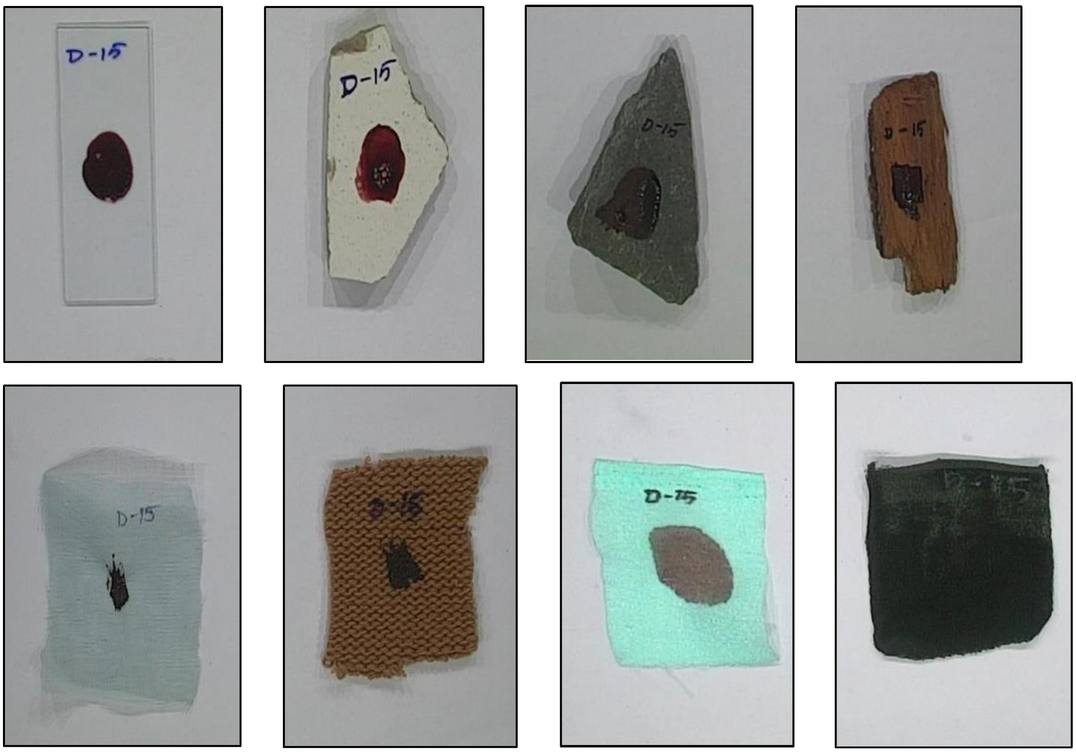
- The stains were observed on surfaces;glass, tile piece,stone & wood and fabrics;Cotton, woolen & silk but not on the velvet fabric.

IR Light



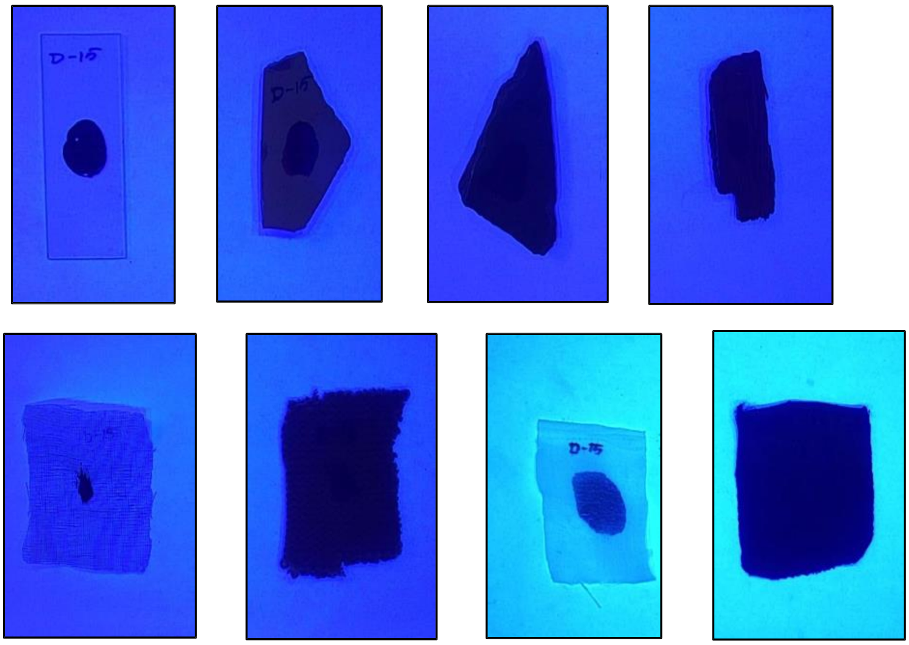
- The stains were observed on surfaces;glass, tile piece & wood and fabrics;cotton, woolen, silk & velvet but on stone surface the only outer ring of the bloodstain were observed.

Day-15 White Light



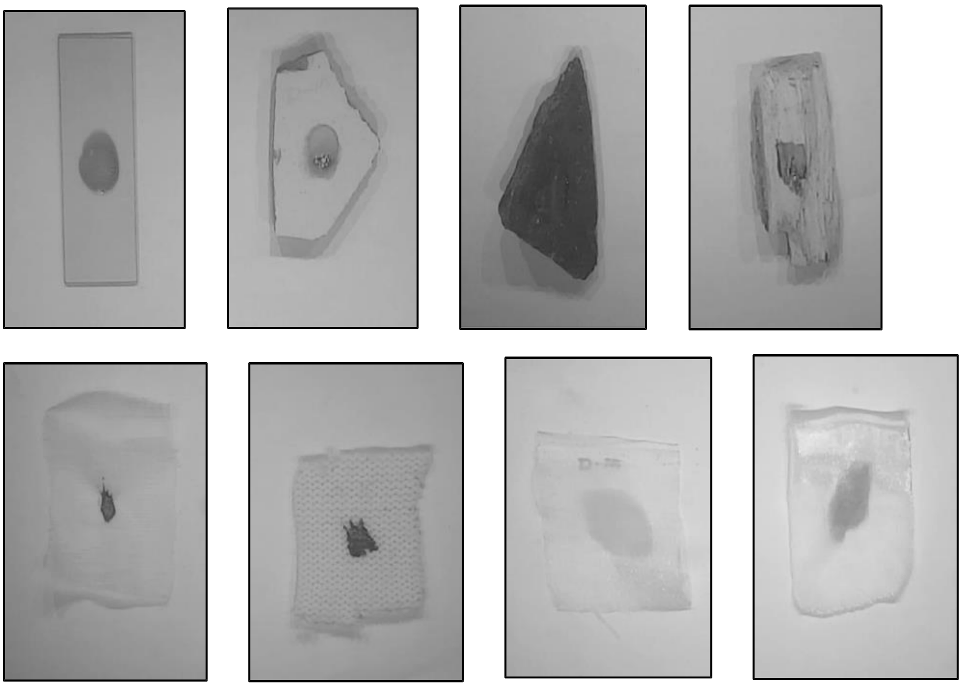
- The reddish stains were observed on surfaces and fabrics , the cracks and scrapings of the bloodstain was observed on glass surface and also on cotton fabric but not on silk, velvet and woolen.

UV Light



- The stains were observed on surfaces; glass,tile piece,stone & wood and fabrics;cotton, woolen & velvet, but not on the velvet fabric.

IR Light



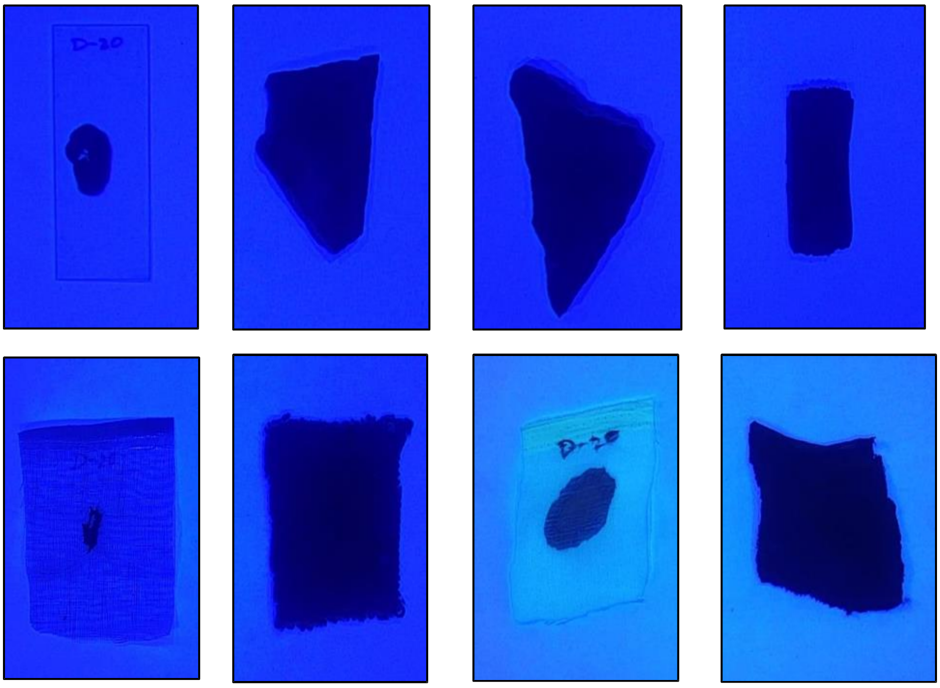
- The stains were observed on the surfaces;glass, tile piece & wood and fabrics; cotton,woolen,silk & velvet, but on stone , there was an presence of outer ring of an bloodstain on the stone surface.

Day-20 White Light

- The bloodstains were observed on surfaces; glass,tile piece,stone & wood and fabrics;cotton,woolen & silk but not on the velvet fabric.

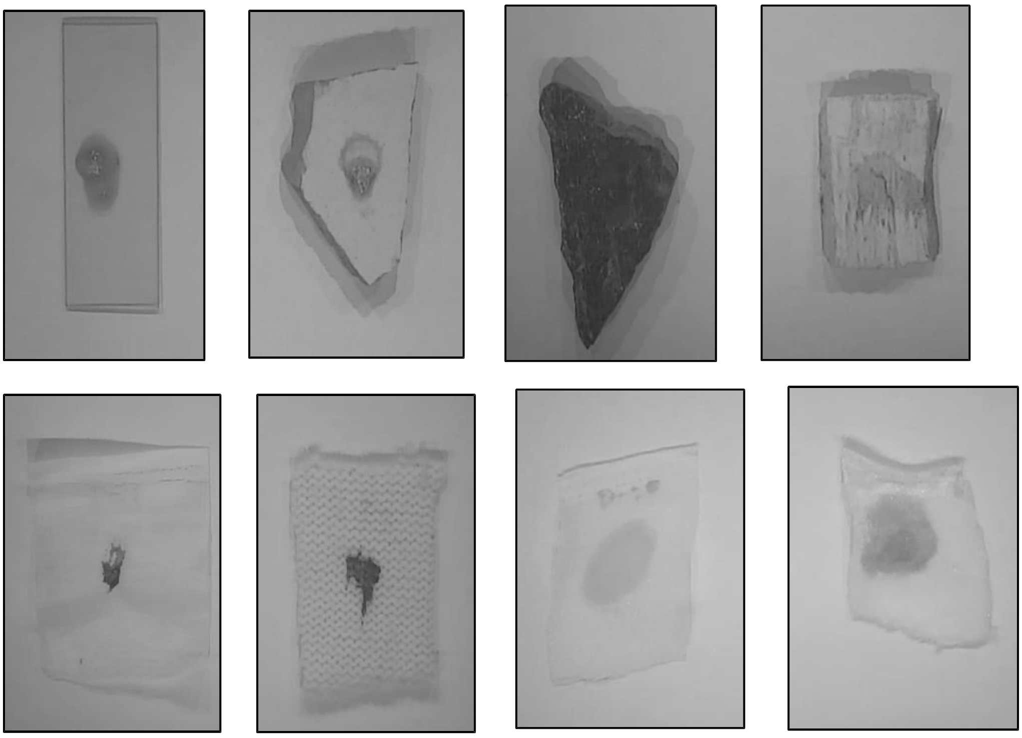
- There was an cracks & scrapings on the glass but only cracks were observed on the stone.

UV Light



- The stains were observed on surfaces;glass, tile piece, stone & wood and fabrics; cotton, woolen & silk but not on the velvet. The cracks and scrapings were observed on the glass surface.

IR Light

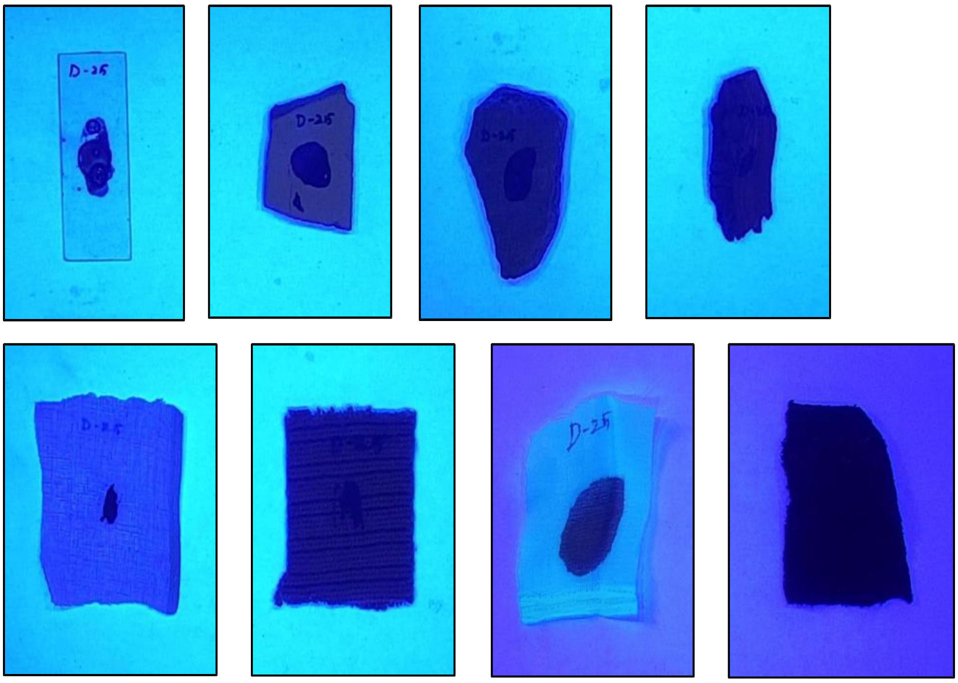


- The stains were observed on the surfaces;glass,tile piece,stone & wood and fabrics;cotton, woolen, silk & velvet under the IR Light source.

Day-25 White Light

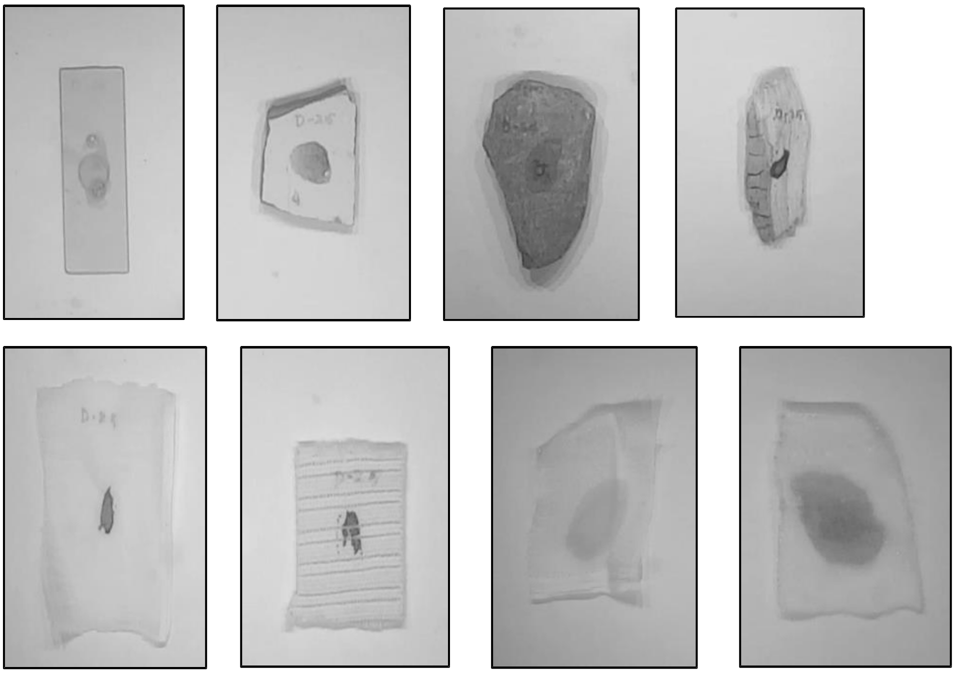
- The stains were observed on the surfaces;glass,tile piece,stone & wood and fabrics;cotton,woolen & silk but except on the velvet fabric.

UV Light



- The stains were observed on the surfaces;glass,tile piece,stone & wood and the fabrics;cotton woolen & silk but except on the velvet fabric under the UV Light

IR Light



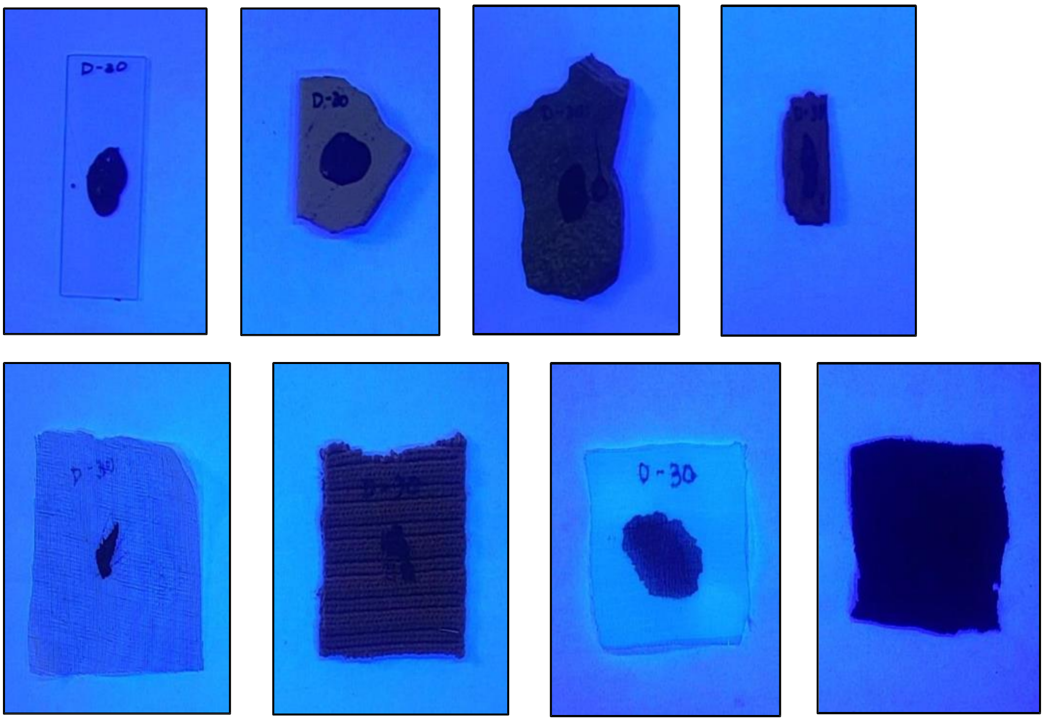
- The stains were observed on surfaces;glass, tile piece, stone & wood and the fabrics;cotton, woolen, silk & velvet under the IR Light Source.

Day-30

White Light

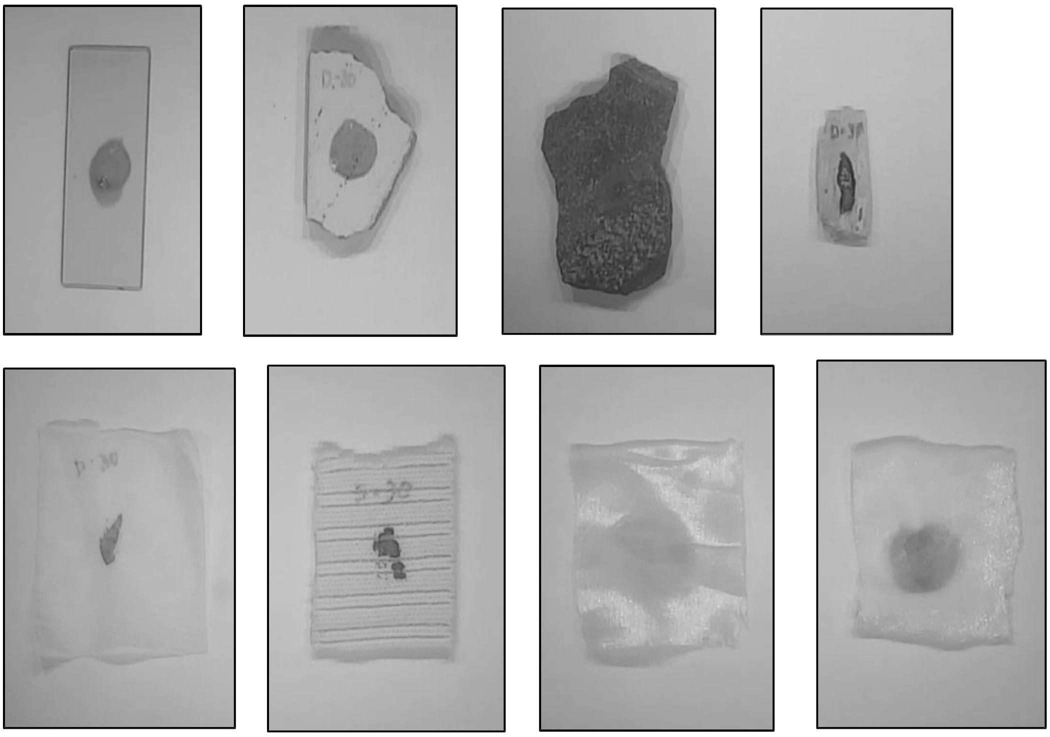
- The stains were observed on surfaces;glass, tile piece,stone & wood and fabrics;cotton, woolen & silk but except on the velvet fabric.

UV Light



- The stains were observed on surfaces;glass, tile piece, stone & wood and the fabrics;cotton,woolen & silk but except on the velvet fabric under the UV Light.

IR Light

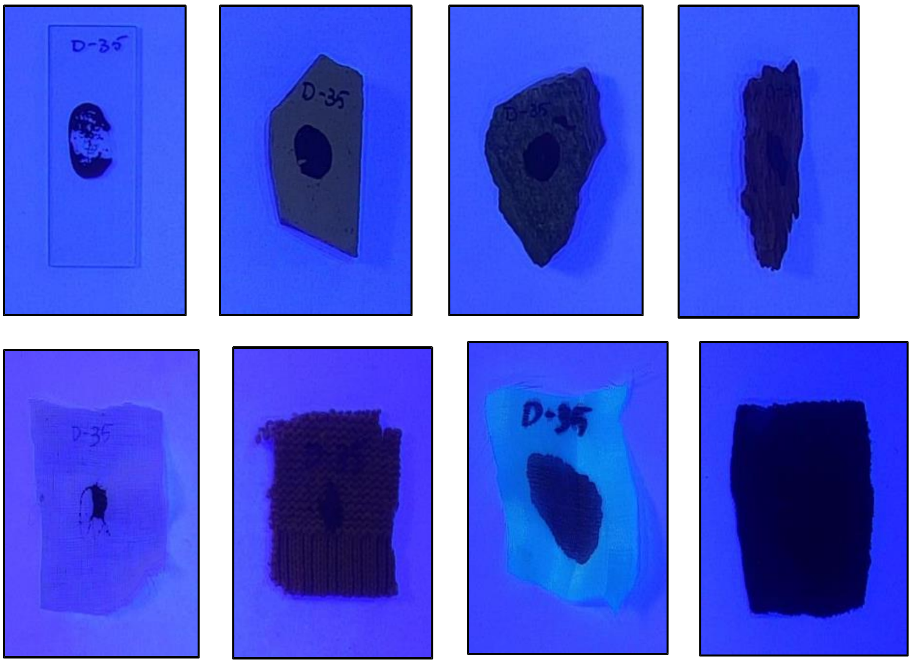


- The stains were observed on surfaces;glass,tile piece, stone & wood and the fabrics;cotton,woolen,silk & velvet under the IR Light.

Day-35 White Light

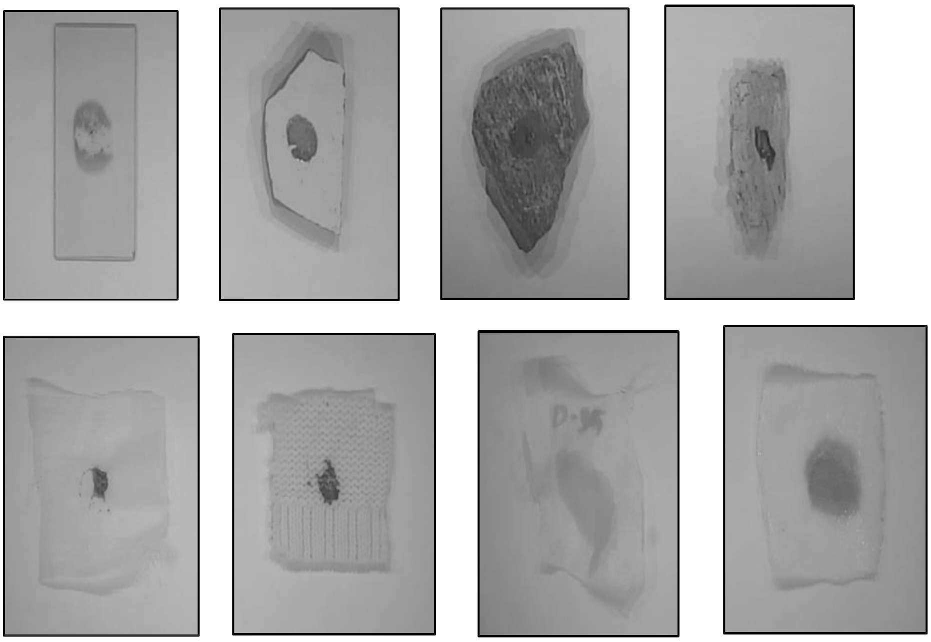
- The stains were observed on surfaces;glass, tile piece, stone & wood and the fabrics;cotton,woolen & velvet but except on the velvet fabric.

UV Light



- The stains were observed on surfaces;glass,tile piece, stone & wood and the fabrics;cotton,woolen & silk but except on the velvet fabric under the UV Light.

IR Light

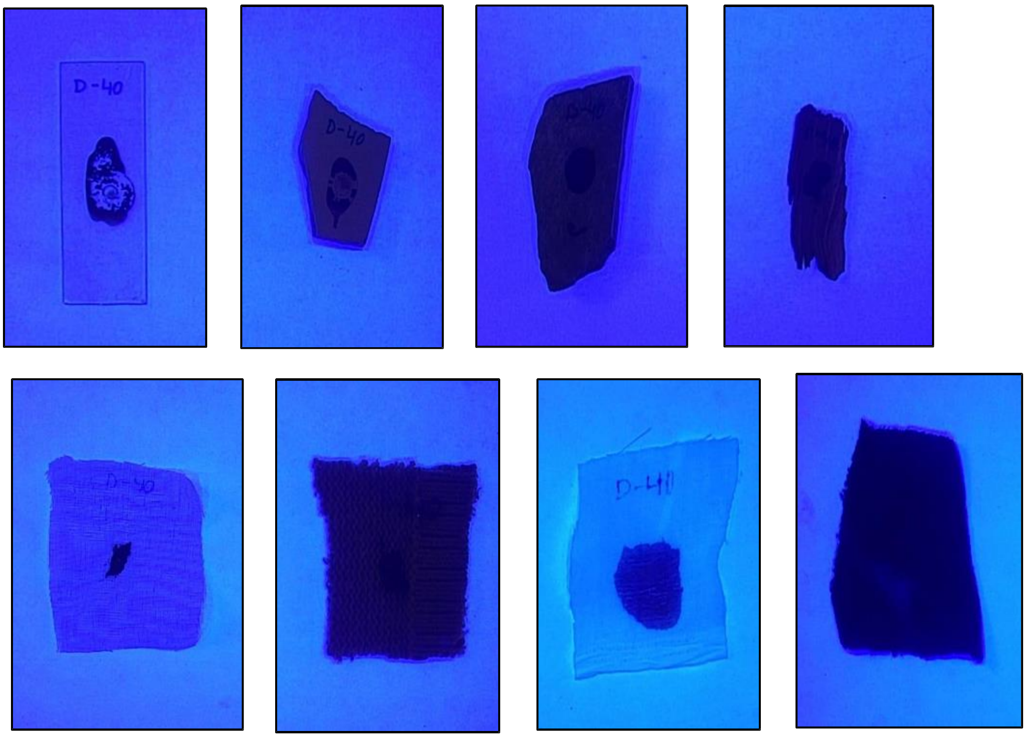


- The stains were observed on surfaces;glass,tile piece,stone & wood and the fabrics;cotton,woolen,silk & velvet under the IR light.

Day-40 White Light

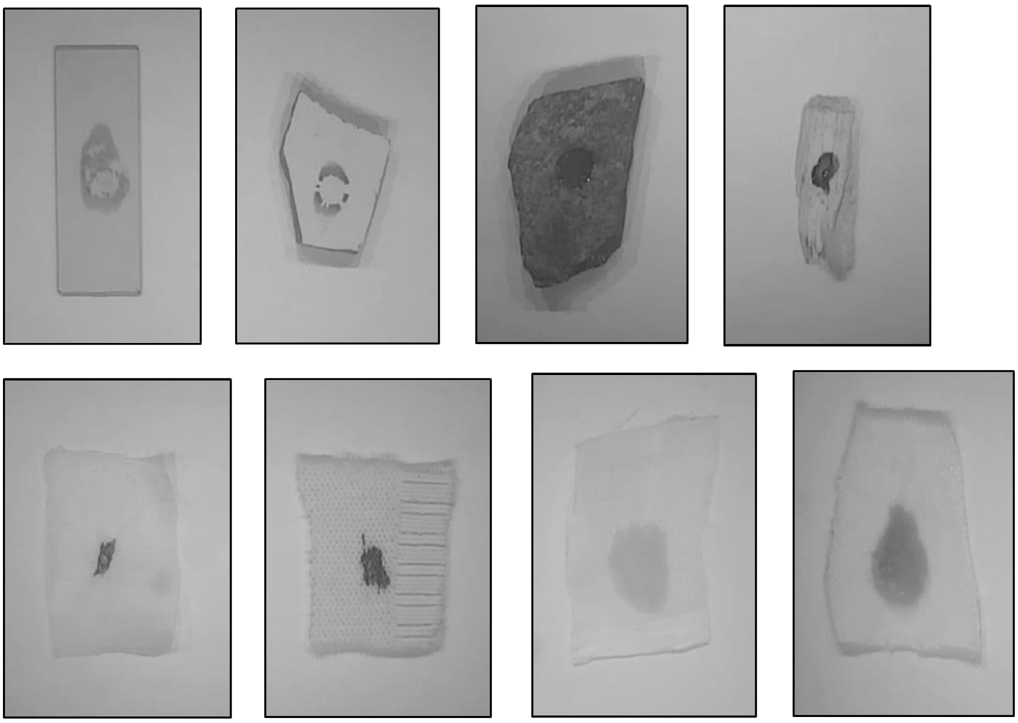
- The stains were observed on surfaces;glass,tile piece,stone & wood and fabrics;cotton,woolen & silk but except on the velvet fabric.

UV Light



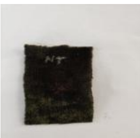
- The stains were observed on surfaces;glass,tile piece,stone & wood and fabrics;cotton,woolen & silk but except on the velvet fabric.

IR Light



- The stains were observed on surfaces;glass,tile piece,stone & wood and the fabrics;cotton,woolen,silk & velvet under the IR Light Source.

Visual Observations:

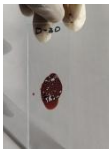
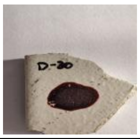
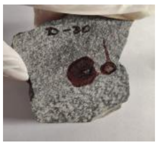
Day	Images	Visual Observations
1-5 (Neat)	Glass Surface 	The reddish stains were observed. There was no change in the size and shape of the bloodstain on the glass surface. There were no cracks and scrapings of the bloodstain on the glass surface.
	Tile Piece Surface 	The reddish stains were observed on the surface. There was no change in the size and shape of the blood and there were no cracks and scrapings on the tile surface.
	Stone Surface 	Reddish stains can be observed on the stone. There was no disturbance of the blood on the stone surface and there were no disturbances of the blood on the stone and there were no cracks and scrapings of the blood on the stone surface.
	Wood Surface 	The reddish stains were observed on the wood surface. There was no change in the shape and size of blood stains on the wood, and there is no cracks or scrapings of the blood on the wood surface.
	Cotton Fabric 	The reddish brown stains were observed. There were no scrapings or cracks of the bloodstain on the cotton fabric.The cotton fabric became harder.
	Silk Fabric 	The reddish stains were observed on the silk fabric . There were no scrapings or cracks of the bloodstains on the silk fabric. The silk fabric became little harder. There was an change in the size and shape of the bloodstain.
	Velvet Fabric 	Brownish stains were observed on the velvet fabric . There were no scrapings or cracks of the bloodstains on the fabric.And the velvet fabric also became harder.
	Woolen Fabric 	The reddish brown stains were observed on the woolen fabric. There were no scrapings or cracks of the bloodstains on the fabric.

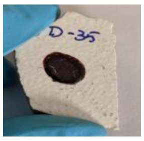
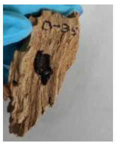
6-10	Glass Surface 	The reddish stains were observed on the glass surface. There was no change of the bloodstain on the glass surface . Few cracks and scrapings were observed on the glass surface.
	Tile Piece Surface 	No change were observed on the tile piece surface. The bloodstain on the tile piece surface as same as on Day -5
	Stone Surface 	No change of the bloodstain on the stone surface. The bloodstain on the stone surface was same as on Day-5. There was no change in the size and shape of the bloodstains.
	Wood Surface 	No change were observed on the wood surface. There was no change in the size and shape of the bloodstains on the wood surface.
	Cotton Fabric 	The reddish brown stains were observed on the cotton fabric. The fabric becomes harder and there was no disturbances of the blood on the cotton fabric.
	Silk Fabric 	The reddish stains were observed on the silk fabric. There was an change in the size and shape of the bloodstain on the silk fabric. The fabric becomes harder.
	Velvet Fabric 	The brownish stains were observed on the velvet fabric. There was no change in the size and shape of the bloodstain on the velvet fabric.
	Woolen Fabric 	The reddish brown stains were observed on the woolen fabric . There was no change in the size and shape of the bloodstain on the woolen fabric.

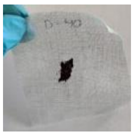
11-15	Glass Surface 	Dark reddish stains were observed on glass surface. Cracks were observed on the whole bloodstain on the glass surface, and scrapings were also been observed.
	Tile Piece Surface 	Dark reddish stains were observed on the tile piece surface. No scrapings or cracks were observed on the tile piece surface.
	Stone Surface 	Dark reddish stains were observed on the stone surface. No cracks or scrapings were observed on the stone surface.
	Wood Surface 	Dark reddish stains were observed on the wood surface. There were no scrapings or cracks of the bloodstain on the wood surface.
	Cotton Fabric 	The reddish brown stains were observed on the cotton fabric. Slight scrapings were observed on the cotton fabric.
	Silk Fabric 	The reddish stains were observed on the silk fabric. There was an change in the size and shape of the bloodstain on the silk fabric. The fabric became harder.
	Velvet Fabric 	The brownish stains were observed on the velvet fabric. There was no change in the size and shape of the bloodstain on the velvet fabric.
	Woolen Fabric 	The reddish brown stains were observed on the woolen fabric . There was no change in the size and shape of the bloodstain on the woolen fabric.

16-20	Glass Surface 	Dark reddish stains were observed on the glass surface. There was an presence of cracks. The scrapings of the bloodstain was coming out of the glass surface.The initial stage of the scrapings were started.
	Tile Piece Surface 	Dark reddish stains were observed on the tile piece surface. No scrapings or cracks were observed on the tile piece surface.
	Stone Surface 	Dark brownish stains were observed on the stone surface. There was an presence of cracks but there were no scrapings on the stone surface.
	Wood Surface 	Dark red or blood red in colour of the stain were observed on the wood surface. There was no cracks or scrapings present on the wood surface.
	Cotton Fabric 	Dark brownish stains were observed on the cotton fabric. There were no cracks but initial stage of scrapings coming out of cotton fabric were observed.
	Silk Fabric 	Dark reddish stains were observed on the silk fabric. There was an change in the size and shape of the bloodstain on the silk fabric.The silk fabric became harder.
	Velvet Fabric 	The brownish stains were observed on the velvet fabric. There was no change observed in the size and shape of the bloodstain on the velvet fabric.
	Woolen Fabric 	The dark brownish stains were observed on the woolen fabric. There were no cracks or scrapings of the bloodstain on the woolen fabric.

21-25	Glass Surface 	Dark reddish stains were on the glass surface. There was an presence of the cracks and scrapings of the bloodstain on the glass surface.
	Tile Piece Surface 	Dark reddish stains were observed on the tile piece surface. There was an presence of cracks and scrapings of the bloodstain on the tile piece were observed.
	Stone Surface 	Dark red in colour stains on the stone surface were observed. The cracks were observed but not the scrapings of the bloodstain on the stone surface.
	Wood Surface 	Dark brownish stains were observed on the wooden surface. There was an presence of cracks on the wood surface but not scrapings of the bloodstain on the wood.
	Cotton Fabric 	Dark brownish stains were observed on the cotton fabric. There were no cracks but initial stage of scrapings coming out of cotton fabric were observed.
	Silk Fabric 	Dark reddish stains were observed on the silk fabric. There was an change in the size and shape of the bloodstain on the silk fabric.The silk fabric became harder.
	Velvet Fabric 	The brownish stains were observed on the velvet fabric. There was no change observed in the size and shape of the bloodstain on the velvet fabric.The fabric became little harder.
	Woolen Fabric 	The dark brownish stains were observed on the woolen fabric. There were no cracks or scrapings of the bloodstain on the woolen fabric.

26-30	Glass Surface 	Dark reddish stains were observed on the glass surface. There was an presence of cracks and scrapings, the scrapings are coming out of the bloodstain on the glass surface.
	Tile Piece Surface 	The dark reddish stains were observed on the tile piece surface. The cracks were observed on the tile piece and also few scrapings coming out of the tile piece surface.
	Stone Surface 	Dark reddish stains were observed on the stone surface. The cracks were observed on the stone surface.
	Wood Surface 	Dark brownish stains were observed on the wood surface. There were the presence of cracks on the wood surface.
	Cotton Fabric 	Dark reddish stains were observed on the cotton fabric. The scrapings of the bloodstain on the cotton fabric were observed.
	Silk Fabric 	Dark reddish stains were observed on the silk fabric. There was an change in the size and shape of the bloodstain on the silk fabric. The silk fabric became harder.
	Velvet Fabric 	The brownish stains were observed on the velvet fabric. There was no change observed in the size and shape of the bloodstain on the velvet fabric.The fabric became little harder.
	Woolen Fabric 	Dark reddish stains were observed on the woolen fabric. The bloodstain look like an resin like material after the passage of 30 days on the woolen fabric.

31-35	Glass Surface 	Dark reddish stains were observed on the glass surface. There was an presence of cracks and scrapings of bloodstain on the surface.The bloodstain scrapings had removed and the centre part became plain with an small rings formation on the glass surface.
	Tile Piece 	The dark reddish stains were observed on the tile piece surface. The cracks were observed on the tile piece and also few scrapings coming out of the tile piece surface.
	Stone Surface 	Dark reddish stains were observed on the stone surface. The cracks were observed on the stone surface and also the initial stage of the scrapings of an bloodstain were observed.
	Wood Surface 	Dark brownish stains were observed on the wood surface. There were the presence of cracks on the wood surface.
	Cotton Fabric 	Dark reddish stains were observed on the cotton fabric. The scrapings of the bloodstain on the cotton fabric were observed.
	Silk Fabric 	Dark reddish stains were observed on the silk fabric. There was an change in the size and shape of the bloodstain on the silk fabric. The silk fabric became harder.
	Velvet Fabric 	The brownish stains were observed on the velvet fabric. There was no change observed in the size and shape of the bloodstain on the velvet fabric.The fabric became little harder.
	Woolen Fabric 	Dark brownish stains were observed on the woolen fabric. The bloodstain look like an resin like material after the passage of 35 days on the woolen fabric.

36-40	Glass Surface 	Dark reddish stains were observed on the glass surface. There was an presence of cracks and scrapings of bloodstain on the surface.The bloodstain scrapings had removed and the centre and the upper part became plain with an small rings formation on the glass surface.
	Tile Piece 	The dark reddish stains were observed on the tile piece surface. The cracks were observed on the tile piece and also the scrapings came out of the tile piece surface and there was an presence of the ring formation on the tile piece surface.
	Stone Surface 	Dark brownish stains were observed on the stone surface. The cracks were observed on the stone surface and also the initial stage of the scrapings of an bloodstain were observed.
	Wood Surface 	Dark brownish stains were observed on the wood surface. There were the presence of cracks on the wood surface.There was no presence of initial stage of bloodstain scrapings on the wood surface.
	Cotton Fabric 	Dark brownish stains were observed on the cotton fabric. The scrapings of the bloodstain on the cotton fabric were observed.
	Silk Fabric 	Dark reddish stains were observed on the silk fabric. There was an change in the size and shape of the bloodstain on the silk fabric. The silk fabric became harder.
	Velvet Fabric 	The brownish stains were observed on the velvet fabric. There was no change observed in the size and shape of the bloodstain on the velvet fabric.The fabric became little harder.
	Woolen Fabric 	Dark brownish stains were observed on the woolen fabric. The bloodstain look like an resin like material after the passage of 40 days on the woolen fabric

Chemical Testing

1. Phenolphthalein Test:

- In phenolphthalein test it showed strong for the glass, tile piece and stone surface.It showed moderate for wood surface and cotton fabric and weak for woolen, silk & velvet fabrics.

O-Tolidine Test:

- In O-Tolidine test it showed strong for glass, tile piece and stone surfraces.It showed moderate for wood surface and cotton, silk and velvet fabrics & weak for the woolen fabric.

Chemical Testing

1. Phenolphthalein Test:

	Phenolphthalein Test		
Surfaces	**Weak**	**Moderate**	**Strong**
Glass			++ (80%)
Tile Piece			++ (80%)
Stone			++ (80%)
Wood		+ (50%)	
Fabrics			
Cotton		+ (50%)	
Silk	+ (25%)		
Velvet	+ (25%)		
Wool	+ (Faint +ve) (15%)		

O-Tolidine Test:

	O-Tolidine Test		
Surfaces	**Weak**	**Moderate**	**Strong**
Glass			+++ (94%)
Tile Piece			++ (90%)
Stone			+++ (96%)
Wood		++(60%)	
Fabrics			
Cotton		++(55%)	
Silk		++(60%)	
Velvet		+++(65%)	
Wool	++(25%)		

Comparative Analysis

1. Surface Retention of Bloodstains:

1. Hard surfaces like glass, tile, and stone demonstrated strong reactions in both Phenolphthalein and O-Tolidine tests, confirming that blood remained detectable even after aging.
2. Wood showed a moderate reaction, likely due to absorption and gradual degradation of blood over time.

2. Fabric Absorption and Stain Breakdown:

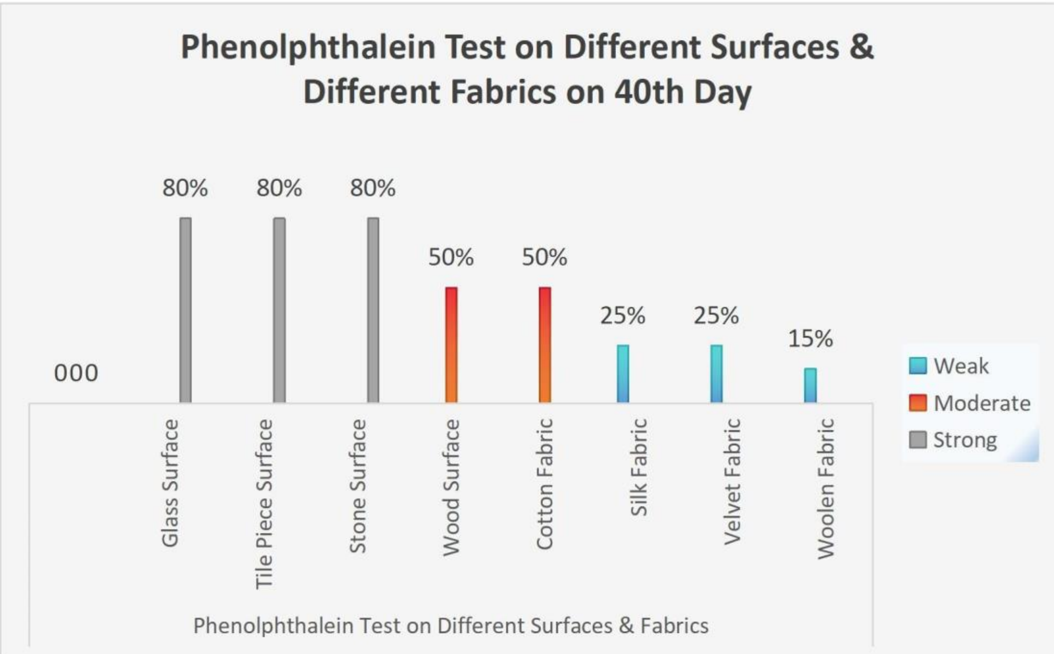
1. Cotton had a moderate reaction in both tests, suggesting retention but with progressive drying and absorption.
2. Silk and Velvet reacted weakly in Phenolphthalein but moderately in O- Tolidine, which could indicate partial degradation or selective chemical reactivity.
3. Wool showed the weakest results, particularly in the Phenolphthalein test, suggesting significant breakdown or absorption of blood over time.

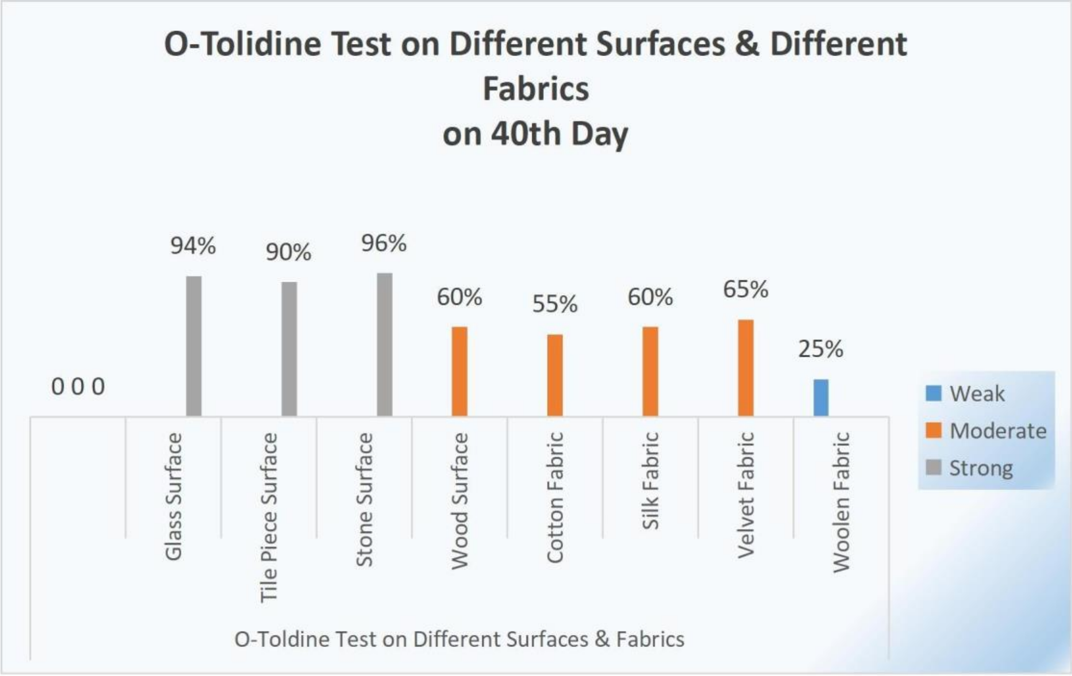
Key Takeaways by the 40th Day:

- Hard Surfaces retained stains well, with minimal degradation.
- Absorbent Fabrics like cotton showed moderate retention but became harder due to blood drying.
- Silk and Velvet exhibited differing chemical reactivity, possibly indicating varying absorption rates.
- Wool demonstrated the weakest reactions, suggesting rapid aging effects leading to diminished detection.

Potential Interpretations:

- **Forensic Significance**: Bloodstains on non-porous surfaces (glass, tile, stone) remain detectable for extended periods, making them ideal for long-term forensic analysis.
- **Challenges on Fabrics**: Highly absorbent fabrics like wool may significantly impact chemical test sensitivity, requiring alternative forensic techniques for older stains.





CHAPTER-5

RESULTS:

Alternate Light Source

Over the course of 40 days, bloodstains were observed on various surfaces (glass, tile, stone, wood) and fabrics (cotton, silk, velvet, woolen) using white light, UV light, and IR light.

Neat Samples (Days 1–5)

- **White Light:** Reddish stains appeared on all surfaces except velvet fabric.
- **UV Light:** Stains were visible on glass, tile, cotton, woolen, and silk, but absent on stone, wood, and velvet fabric.
- **IR Light:** Bloodstains were detected on all materials except stone.

10th Day

- **White Light:** Stains remained similar to neat samples, but cracks and scrapings appeared on glass.
- **UV Light:** Stains were observed on all surfaces and fabrics except velvet fabric.
- **IR Light:** Outer ring of the bloodstain became visible on stone, while other surfaces remained unchanged.

15th Day

- **White Light:** Bloodstains remained visible on all surfaces and fabrics except velvet fabric. Cracks and scrapings appeared on glass and cotton fabric.
- **UV Light & IR Light:** No significant changes from the 10th day.

20th Day

- **White Light:** Cracks appeared on stone surface, with intensified scrapings on glass.
- **UV Light:** Similar to the 15th-day observations, but additional cracks and scrapings noted on the glass surface.
- **IR Light:** Stains persisted across all surfaces and fabrics.

25th Day

- **White Light, UV Light:** No significant changes from the 20th day.
- **IR Light:** The stains were observed on all the surfaces & the fabrics.

30th Day

- **White Light:** Cracks and scrapings additionally appeared on the tile surface.
- **UV Light & IR Light:** No noticeable variations.

35th Day

- **White Light:** Cracks formed on stone surface.
- **UV Light & IR Light:** Observations remained consistent.

40th Day

- **White Light:** Cracks appeared on the wood surface.
- **UV Light & IR Light:** Observations remained unchanged.

Conclusion

- **White Light:** Cracking and scraping intensified over time, especially on glass, tile, stone, and wood.
- **UV Light:** Bloodstains remained visible on most surfaces except on the velvet fabric.
- **IR Light:** Bloodstains were consistently detectable on all surfaces except stone till 20th day , later the bloodstain became darker & it was detected in the IR Light on the stone surface.

Visual Observations:

Surface Observations

The observations were made on various surfaces, including glass, tile, stone, and wood, over a period of 40 days.

- **Glass surface:** Initially, there were no changes observed in the bloodstain. However, after 10-15 days, cracks and scrapings were observed. By 30-40 days, the bloodstain scrapings had removed, and a ring formation was observed.
- **Tile surface:** No changes were observed initially. However, after 20-25 days, cracks were observed, and by 30-40 days, scrapings and ring formation were observed.
- **Stone surface:** No changes were observed initially. However, after 20-25 days, cracks were observed, and by 30-40 days, the initial stage of scrapings was observed.
- **Wood surface:** No changes were observed initially. However, after 20-25 days, cracks were observed, but no scrapings were observed.

Fabric Observations:

The observations were made on various fabrics, including cotton, silk, velvet, and woolen, over a period of 40 days.

- **Cotton fabric:** The fabric became harder, and scrapings were observed after 15-20 days.
- **Silk fabric:** There was a change in the size and shape of the bloodstain throughout the observation period, and the fabric became harder.
- **Velvet fabric:** There was no change observed in the size and shape of the bloodstain, but the fabric became slightly harder.
- **Woolen fabric:** The bloodstain resembled a resin-like material after 30-40 days.

Chemical Testing:

Phenolphthalein Test:

- **Strong reactions:** Glass, tile, stone surfaces.
- **Moderate reactions:** Wood, cotton.
- **Weak reactions:** Wool, silk, velvet fabrics.

O-Tolidine Test:

- **Strong reactions:** Glass, tile, stone surfaces.
- **Moderate reactions:** Wood, cotton, silk, velvet.
- **Weak reaction:** Wool.

Combined Findings:

1. **Surface Types:**
   - Hard, non-porous surfaces (glass, tile, stone) retained detectable bloodstains well and showed strong reactions consistently in both tests. These surfaces are ideal for forensic analysis due to minimal degradation over time.
   - Wood exhibited moderate reactions in both tests, suggesting some retention but with effects of absorption and aging.

2. **Fabric Types:**
   - **Cotton:** Moderate retention and reactivity in both tests, indicating drying and partial absorption over time.
   - **Silk & Velvet:** Weak Phenolphthalein reactions but moderate O-Tolidine responses point to selective reactivity or partial degradation.
   - **Wool:** Weakest reactions overall, indicating rapid stain breakdown and low detection sensitivity after aging.

Key Observations by the 40th Day:

- **Hard Surfaces:** Excellent bloodstain retention with minimal impact from aging.
- **Cotton:** Moderate retention with drying effects reducing stain fluidity.
- **Silk & Velvet:** Reactivity differences indicate variability in stain absorption and breakdown.
- **Wool:** Significant degradation reduces detection effectiveness over time.

Forensic Implications:

- **Non-Porous Surfaces:** Glass, tile, and stone are reliable for long-term evidence collection due to their strong stain retention.
- **Fabrics:** Wool poses challenges for forensic detection due to rapid absorption and aging, while cotton, silk, and velvet vary in sensitivity and chemical reactivity, highlighting the need for forensic approaches to analyze older stains.

CHAPTER-6

DISSCUSSION:

This study investigated the time-dependent degradation of bloodstains across four surfaces (glass, tile piece, stone, and wood) and four fabrics (cotton, silk, velvet, and woolen) using a combination of visual observation, alternate light sources (UV and IR), and chemical presumptive tests (o-tolidine and phenolphthalein) over a period of 40 days. The goal was to simulate realistic forensic conditions and evaluate how substrate type and stain age influence the detectability and diagnostic reliability of each method.

Visual Observation: Bloodstains on porous surfaces like wood and stone underwent rapid drying and oxidation, leading to earlier browning and flaking by Day 20. In contrast, on non- porous surfaces like glass and tile pieces, stains remained darker and more stable for longer periods, retaining visible color even up to Day 30. These trends are consistent with findings from Zadora and Mioduszewski (2007) and Hesse et al. (2013), who reported that non- absorbent surfaces slow the oxidation of hemoglobin due to reduced exposure to air and microbial activity.

Fabrics presented distinct behavior. Cotton and woolen fabrics, due to their absorbent nature, exhibited clear and predictable aging patterns. On the other hand, silk and velvet, particularly velvet due to its dense pile, retained stain coloration longer, masking aging cues. Similar behavior was reported by de Castro and Rando (2016), though this study is unique in combining these fabrics in a single experimental framework with long-term aging.

Alternate Light Sources: The use of UV light (365 nm) proved highly effective in enhancing stain visibility, especially between Days 10–30. UV illumination was particularly helpful on complex backgrounds like velvet and on porous surfaces where visual contrast diminished with time. This supports work by Coyle et al. (2005) and Khurana et al. (2017), who emphasized UV's usefulness in identifying latent and aged stains due to hemoglobin byproducts such as porphyrins. In contrast, IR light was found less reliable overall, showing only mild improvement in contrast, mainly on darker surfaces.

A unique contribution of this study is the integration of all three methods across eight substrates under uniform conditions, revealing how each technique performs over time. Compared to past studies that often focused on a single surface or method, this broader approach helps create a practical model for forensic application. Notably, this study results highlight that visual and UV-based observations were more reliable than chemical tests beyond Day 20, while substrate characteristics like porosity and texture significantly influenced stain aging and detectability.

Chemical Test Comparison (O-Tolidine vs. Phenolphthalein):

A major and unique contribution of this study is the detailed, side-by-side evaluation of o- tolidine and phenolphthalein across substrates and time. While both are standard presumptive tests, o-tolidine showed superior performance, yielding strong positive reactions on many materials even beyond Day 20–25. This contrasts sharply with phenolphthalein, which generally stopped producing clear color changes after Day 15–20, especially on smooth and non-absorbent surfaces such as silk and tile.

This finding extends the work of Rao and Rao (2019), who briefly noted o-tolidine's higher sensitivity but did not analyze its performance over an extended time or on multiple substrates. Moreover, unlike Barni et al. (2005) who focused on fresh stains, in this study uniquely explored long-term reactivity and color intensity. O-tolidine's blue-green coloration was easier to detect on faded or darkened stains, while phenolphthalein's pink response was often too weak to register visually on aged or dark materials.

These results demonstrate that o-tolidine is more effective for detecting aged blood, making it especially useful in forensic scenarios involving older or dried stains. While phenolphthalein is widely used due to its lower toxicity and better specificity, the risk of false negatives in aged samples makes o-tolidine a valuable complementary reagent.

Novelty in Experimental Design:

This research is also unique in the variety and combination of materials tested, extending to both architectural surfaces (tile, stone, glass, wood) and textile types (including less commonly studied velvet and wool). Furthermore, testing was extended to Day 40, whereas most prior research limits observations to 10–21 days. This extended timeline provides practical forensic insights for delayed crime scene investigations or cold cases.

CHAPTER-7

CONCLUSION:

This study provides a comprehensive analysis of bloodstain aging on various surfaces (glass, tile, stone, and wood) and fabrics (cotton, silk, velvet, and woolen) using visual inspection, alternate light sources (UV and IR), and two chemical tests (o-tolidine and phenolphthalein) over a 40-day period. The results demonstrate that surface porosity and material composition significantly influence stain appearance and detectability over time. Visual changes were most prominent on porous substrates like wood and fabrics like cotton, while non-porous surfaces like glass and tile retained clearer stains for longer durations.

The use of UV light enhanced visibility of older stains, especially on absorbent or textured materials, whereas IR was less effective overall. Among chemical tests, o-tolidine proved to be more sensitive and reliable than phenolphthalein, detecting blood in older stains where phenolphthalein failed, particularly after Day 20. This highlights o-tolidine's potential as a superior reagent in delayed or cold-case investigations.

The study's unique combination of extended aging, varied substrates, and multi-method testing contributes valuable insights to forensic science, particularly in estimating the time since deposition of bloodstains. Future research should incorporate environmental variability, different biological sources, and advanced biochemical assays to further refine age estimation models.

CHAPTER-8

BIBLIOGRAPHY:

1. Andrasko, Jan. (1997). *The Estimation of Age of Bloodstains by HPLC Analysis*.
2. Bremmer, Rolf H., et al. (2011). *Age Estimation of Bloodstains by Hemoglobin Derivative Determination Using Reflectance Spectroscopy*.
3. Ramsthaler, Frank, et al. (2012). *Drying Properties of Bloodstains on Common Indoor Surfaces*.
4. Edelman, Gerda, et al. (2012). *Identification and Age Estimation of Bloodstains on Colored Backgrounds by Near Infrared Spectroscopy*.
5. Sun, Baozhong, et al. (December 2015). *Study on the Relations Between Bloodstain Age on Cotton Fabric with Color and Color Difference*.
6. Li, Jingyao, et al. (2016). *Alternative Method for Determining the Original Drop Volume of Bloodstains on Knit Fabrics*.
7. Edelman, Gerda J., et al. (2016). *Practical Implementation of Bloodstain Age Estimation Using Spectroscopy*.
8. Doty, Kyle C., et al. (2017). *Predicting the Time of the Crime: Bloodstain Aging Estimation for Up to Two Years*.
9. Di Lascio, A., et al. (2018). *Investigating the Color of Bloodstains on Archaeological Cloths: The Case of the Shroud of Turin*.
10. Choi, Wooseok, et al. (2019). *Highly Sensitive & Accurate Estimation of Bloodstain Age Using Smartphone*.
11. Wetzel, Will. (September 16, 2024). *Determining the Age of Bloodstains at Crime Scenes Using ATR FT-IR Spectroscopy and Chemometrics*.
12. Russell, Cenya, et al. (2025). *The Persistence of Bloodstains on Buried Fabrics & Robustness of the Leucomalachite Green Test in Detecting Aged Bloodstains on Buried Fabrics*.