ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2200

Evaluation of Microbial Growth in Dried Blood Stains

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Publication Date: 2025/05/15

Abstract; Blood is one of the most critical type of biological fluid recovered from crime scene and often crucial in reconstructing events, identifying individuals and time since deposition. This study focuses on the microbial aspect of the blood as blood provide an ideal environment for microbial growth due to its nutrient content. Microbial growth in dried bloodstains occurs in distinct phases and is influenced by environmental conditions such as temperature, humidity and exposure to air. The suggested approach is experimental, requires blood samples that are deposited on any surface and collected at various time intervals (6, 24, 48, 72, 96 hours). The study aims to investigate microbial growth dynamics in bloodstains over time and their impact on the spectral properties measured through UV-Vis spectrophotometer. The turbidometeric analysis measured the absorbance of rehydrated sample at 600nm to quantify microbial proliferation. This approach bridges forensic science and microbiology, ensuring a foundation for further researches in future.

Keywords; *Bloodstains; Microbial Growth; Incubate; Centrifuge; Absorbance; Time Intervals.*

How to Cite: Aneena Wahab; Sreya Menon; Anjali Anne Jacob (2025). Evaluation of Microbial Growth in Dried Blood Stains. *International Journal of Innovative Science and Research Technology*, 10(4), 3750-3753. https://doi.org/10.38124/ijisrt/25apr2200

I. INTRODUCTION

Bloodstains are one of the most frequently encountered type of forensic evidence in crime scene. Microorganisms naturally interact with blood, and this study is designed to examine the microbial growth in dried bloodstains over time. Despite the known presence of microorganisms in blood, limited research has focused on how microbes grow in dried blood stains. Studying the microbial proliferation in dried blood stains helps in understanding biological changes that occur after deposition. Microbial contamination can affect forensic analysis, including DNA profiling and bloodstain pattern interpretation. In this study blood is studied from a microbial perspective focusing on observing microbial growth in dried bloodstains over time. Blood samples were deposited on the surface in a common room and are allowed to dry naturally for the simulation of real-world forensic scenarios. Over specific time intervals (6, 24, 48, 72, 96 hours), dried bloodstains were systematically scraped out, processed with distilled water, incubated, and centrifuged. The supernatant was then analysed using UV-Vis spectrophotometery to observe microbial growth rates. UV-Vis spectrophotometer measures variations in absorbance that indicate microbial presence and how it changes with time.

➢ *Objectives*

- To develop and validate a method to understand microbial growth patterns.
- To identify whether microbial species increase in blood stains over time.
- To analyze microbial succession rate under laboratory controlled conditions.

➢ *Hypothesis*

- The rate of microbial growth varies over time in a controlled environment.

II. METHODOLOGY

➢ *Materials Required*

Distilled water, Sterile swabs, Blood samples, Sterile centrifuge tubes, Sterile pipettes and tips, Biosafety cabinet, Incubator, Eppendorf Centrifuge 5424R and Shimadzu UV-1900i UV-Vis spectrophotometer.

➢ *Method*

Estimating Microbial Growth Using UV-Vis spectrophotometer

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- Blood samples were deposited on glass surface and analysed at various time intervals under real time environmental condition.
- Take 2ml of distilled water into a centrifuge tube and add small amount of scraped dried bloodstains. Ensure the dried stains were scraped using a sterile tool.
- The sample was incubated at 37c for 30 minutes to 1 hour with gentle shaking to facilitate rehydration.
- The rehydrated sample was centrifuged at 2000 RCF for 10 minutes in the Eppendorf Centrifuge 5424R to pellet large debris.
- The supernatant was carefully transferred to a fresh sterile tube (To confirm microbial growth in dried bloodstains, the supernatant obtained after centrifugation was swabbed and streaked onto an agar plate. The plates were then incubated overnight under controlled conditions to assess colony formation, allowing for further validation of microbial proliferation over time).
- A second centrifugation was performed at a higher speed (3000-5000 RCF) for 10 minutes to further clarify the sample.
- Turbidity was assessed to evaluate microbial presence. The absorbance of the samples was measured at 600 nm using a spectrophotometer. Absorbance measurements were taken at 6, 24, 48, 72, and 96 hours using the Shimadzu UV-1900i UV - Vis spectrophotometer at a wavelength of 600 nm for bacterial proliferation detection. The 600 nm wavelength is commonly used for measuring bacterial optical density (OD) because it allows accurate detection of cell growth without causing harm to bacteria, unlike UV wavelengths.
- Before measuring the absorbance, a blank calibration was performed using distilled water in the UV-Vis spectrophotometer to ensure accuracy in the readings.
- The values obtained were used to track microbial colonization and growth trends. Values of absorbance for the different time intervals were performed in triplicate.

III. RESULT ANALYSIS

➢ *Tables and Figures*

Table 1 Absorbance Measurements (600nm) using UV-Vis spectrophotometer.

TIME INTERVALS	MEAN ± SD
6	0.16267±0.041
24	1.0883±0.679
48	0.576±0.160
72	0.869±0.145
96	0.5367±0.124

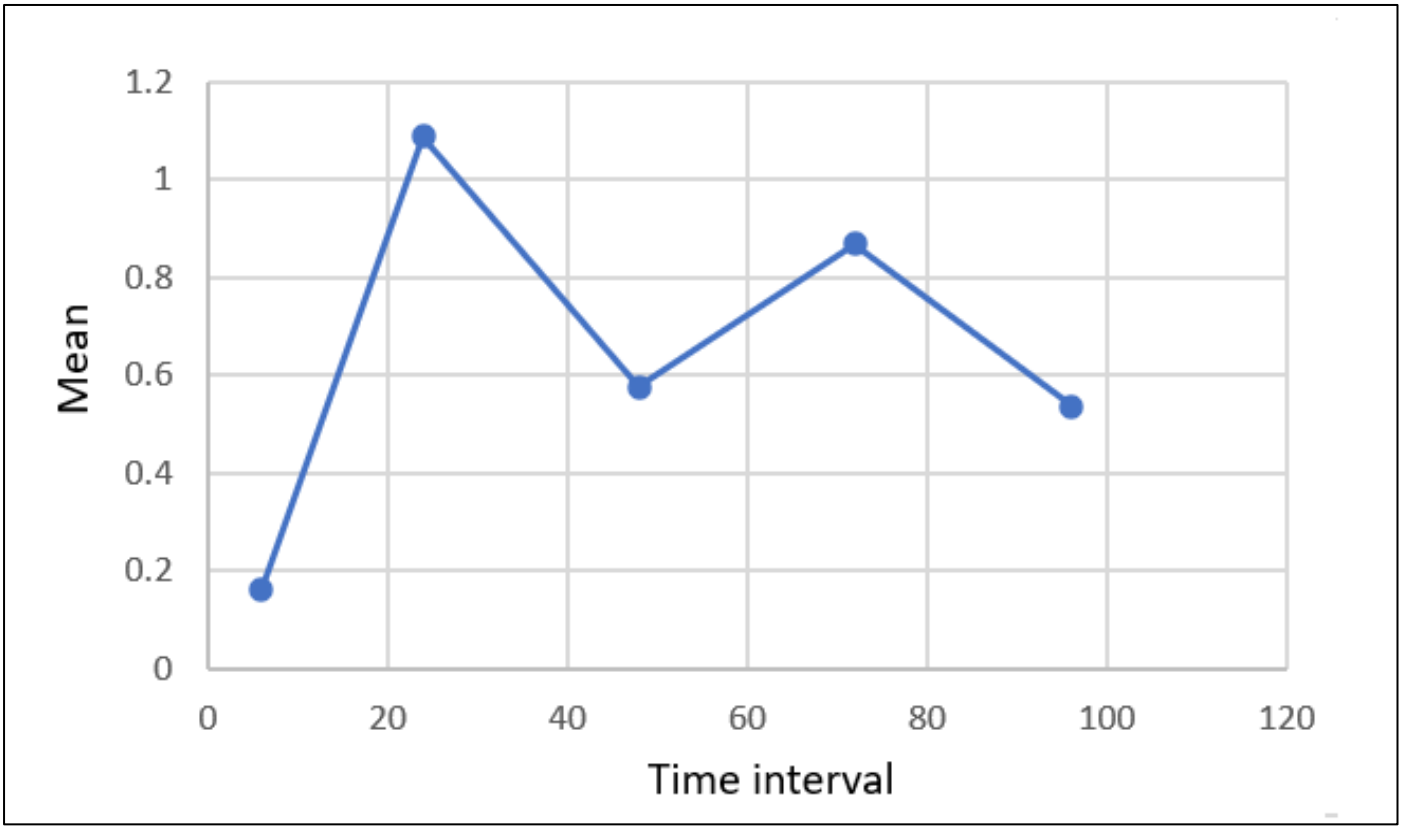

Fig 1 Graph Showing the Absorbance Measurements.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2200

➢ *Major Findings*

- *Measurement Using UV-Vis spectrophotometer*

The absorbance values, indicative of microbial proliferation, fluctuated over time, reflecting changes in microbial population dynamics. To confirm microbial growth, the supernatant was streaked onto an agar plate, which exhibited microbial colonies. In UV-Vis Spectrophotometer, a blank calibration with distilled water showed 0.00 absorbance. At 6 hours, absorbance values were 0.164, 0.213, and 0.121, indicating the presence of microbial activity. At 24 hours, the absorbance values suggested an initial increase in microbial colonization, with recorded values of 0.712, 1.873, and 0.680. This phase likely represents the early growth stage, where microorganisms begin utilizing the available nutrients in the bloodstain. The temperature at this time was 27.4°C, and the humidity was 57%.

By 48 hours, a decrease in absorbance indicated a potential decline in microbial activity. This reduction may have resulted from environmental stressors, depletion of readily available nutrients, or a transition to the stationary phase of microbial growth. The absorbance values recorded were 0.633, 0.700, and 0.395. The temperature remained at 27.4°C, while the humidity slightly increased to 58%. At 72 hours, an increase in absorbance was observed, suggesting a resurgence in microbial growth. This phase may be attributed to the proliferation of secondary colonizers or microbial adaptation to the bloodstain environment, allowing for sustained metabolic activity. The absorbance values recorded at this stage were 1.023, 0.850, and 0.734. The temperature was 27.6°C, and the humidity rose to 60%. This resurgence could be due to the breakdown of complex organic components within the bloodstain, providing an alternative nutrient source for microbial communities.

By 96 hours, the absorbance values showed a decline in microbial activity, with recorded values of 0.545, 0.409, and 0.656. This decrease may be attributed to nutrient exhaustion, biofilm formation, or microbial competition, leading to stabilization or the death of certain microbial populations. At this stage, the temperature increased to 28.2°C, while the humidity remained at 60%. Overall, the trends observed in absorbance values suggest a dynamic microbial colonization pattern on the bloodstains over time.

IV. DISCUSSION RELATED TO HYPOTHESIS

With respect to hypothesis, the UV-Vis spectrophotometer absorbance values provide quantitative evidence of microbial growth trends over time. At 24 hours, the results indicated the early colonization phase, consistent with prior studies showing initial bacterial growth on bloodstains. By 48 hours, a decline in microbial activity was observed, suggesting potential environmental stressors or competition. At 72 hours, a resurgence in microbial growth was noted, which may indicate secondary colonization due to the breakdown of complex organic components, providing alternate nutrient source for the existing microbes. However, by 96 hours, microbial activity declined again, possibly due to nutrient depletion or biofilm maturation leading to growth stabilization. The absorbance values recorded indicate microbial growth is not constant but follows a fluctuating pattern over time.

➢ *Limitations*

A key limitation of this study was the short timeframe, which prevented in-depth analysis and further investigation into the procedure's potential. The procedure was conducted under controlled conditions, the result may not fully represent real-world scenarios. Factors such as temperature, humidity and other environmental factors in an uncontrolled setting can influence microbial growth and absorbance readings differently. In real crime scenes, pre-existing microbes in the blood may alter microbial growth patterns

V. CONCLUSION

The study conducted observed microbial growth in dried bloodstains over time by measuring absorbance using UV-Vis spectrophotometry at 600 nm. The results showed variations in microbial activity across different time intervals. At 6 hours, the dried sample indicated the presence of microbes. At 24 hours, the absorbance was highest, indicating significant microbial presence. At 48 hours, it dropped, suggesting a decline in microbial growth. However, at 72 hours, the absorbance increased again, possibly due to microbial adaptation or secondary growth. By 96 hours, the absorbance decreased, which could indicate depletion of nutrients or unfavorable conditions for further microbial proliferation. These fluctuations highlight the variations of microbial growth in dried bloodstains, which may be influenced by factors such as nutrient availability and environmental conditions.

Overall, this study demonstrates that microbial growth in dried bloodstains is time-dependent and varies under different conditions. The findings contribute to forensic science by providing insights into how microbial succession in bloodstains may be used for post-deposition interval estimation or crime scene reconstruction.

REFERENCES

[1]. Quraishi R, Lakshmy R, Mukhopadhyay AK, Jailkhani BL. Analysis of the stability of urea in dried blood spots collected and stored on filter paper. Ann Lab Med. 2013 May;33(3):190-2. doi: 10.3343/alm.2013.33.3.190. Epub 2013 Apr 17. PMID: 23667845; PMCID: PMC3646193.

[2]. Robinson JM, Pasternak Z, Mason CE, Elhaik E. Forensic Applications of Microbiomics: A Review. Front Microbiol. 2021 Jan 13;11:608101. doi: 10.3389/fmicb.2020.608101. PMID: 33519756; PMCID: PMC7838326.

[3]. Nodari R, Arghittu M, Bailo P, Cattaneo C, Creti R, D'Aleo F, Saegeman V, Franceschetti L, Novati S, Fernández-Rodríguez A, Verzeletti A. Forensic Microbiology: When, Where and How. Microorganisms. 2024 May 14;12(5):988.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2200

[4]. Dash HR, Das S. Microbial degradation of forensic samples of biological origin: potential threat to human DNA typing. Molecular biotechnology. 2018 Feb;60(2):141-53.
[5]. Gouello A, Dunyach-Remy C, Siatka C, Lavigne JP. Analysis of Microbial Communities: An Emerging Tool in Forensic Sciences. Diagnostics (Basel). 2021 Dec 21;12(1):1. doi: 10.3390/diagnostics12010001. PMID: 35054168; PMCID: PMC8774847.
[6]. Speruda M, Piecuch A, Borzęcka J, Kadej M, Ogórek R. Microbial traces and their role in forensic science. J Appl Microbiol. 2022 Apr;132(4):2547-2557. doi: 10.1111/jam.15426. Epub 2022 Jan 5. PMID: 34954826.
[7]. Huntress A. OD600 Measurements: An Easy Guide for Researchers Using Bacterial Cultures: bitesizebio.