

## Body Fluid Aging and Time Estimation

Lissum Yabu

**How to cite this article:***Lissum Yabu*. Body Fluid Aging and Time Estimation. *Int Jr of Forensic Sci*. 2025; 8(1): 41-44**ABSTRACT**

A crucial component of forensic serology is determining the age of biological fluids found at crime scenes, which provides information on the chronology of criminal activity. Reconstructing events and supporting alibis are made easier with accurate time determination, which has a big influence on court cases. Developments in biochemical, molecular, and spectroscopic methods over time have opened up new ways to comprehend how bodily fluids including blood, saliva, semen, and perspiration change over time. The current approaches are examined in this review, including oxidative stress markers, protein and RNA degradation, enzymatic degradation, and newly discovered molecular biomarkers. The sensitivity and dependability of methods like spectroscopy, mass spectrometry, and forensic proteomics in determining stain age have been emphasized. These methods do not, however, come without difficulties, including biological factors, substrate interactions, and environmental impacts. variability, which may make aging projections less accurate.

In order to improve accuracy and consistency, this study also explores the shortcomings of current approaches and suggests potential future possibilities, such as multi-modal analysis and artificial intelligence. The study concludes by highlighting the necessity of internationally recognized procedures to lessen variation in forensic examinations. In order to lay the groundwork for future developments in this crucial field of forensic science, this study attempts to compile the current status of research on body fluid aging and time estimation.

**KEYWORDS**

• Biological stains • Molecular biomarkers • Proteomics • Spectroscopy • Forensic serology • Time estimation • Bodily fluid aging

---

**AUTHOR'S AFFILIATION:**

Student, Department of Forensic Science, Vivekananda Global University, Jaipur, Rajasthan 303012, India.

**CORRESPONDING AUTHOR:**

**Lissum Yabu**, Student, Department of Forensic Science, Vivekananda Global University, Jaipur, Rajasthan 303012, India.

**E-mail:** lissumyabu81@gmail.com

➤ **Received:** 06-02-2025 ➤ **Accepted:** 26-03-2025



Creative commons non-commercial CC BY-NC: This article is distributed under the terms of the creative commons attribution non-commercial 4.0 License (<http://www.creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the Red Flower Publication and Open Access pages (<https://rfppl.co.in>)

## INTRODUCTION

Through the analysis of biological fluids such as blood, semen, saliva, and perspiration, forensic serology plays a crucial part in criminal investigations. Estimating the period since deposition of these fluids is one of its crucial features, providing important information about the chronology of events at a crime scene<sup>1</sup>. Understanding the chemical and molecular changes that take place throughout time and connecting them to a particular time frame is necessary to comprehend the process of bodily fluid aging<sup>2</sup>. In situations like homicides, assaults, and sexual offenses, where the chronology of the evidence deposition is disputed, these estimations are essential<sup>3</sup>.

The pursuit of determining the age of bodily fluids began with early forensic investigations that concentrated on the visual and textural alterations in bloodstains<sup>4</sup>. The first approaches relied on subjective and imprecise macroscopic observations<sup>5</sup>. Accuracy was greatly increased with the introduction of enzymatic degradation markers with the development of biochemical analysis<sup>6</sup>. Time estimation in forensic science has been revolutionized by recent developments that have incorporated molecular approaches like proteome profiling and RNA degradation<sup>7,8</sup>.

Following deposition, biological fluids experience a number of predictable modifications that are impacted by oxidative stress, enzymatic activity, and environmental interactions<sup>9,10</sup>. For example, RNA molecules in saliva and semen degrade in a sequence that corresponds with time, but hemoglobin in blood shows progressive deterioration over time<sup>11,12</sup>. Modern forensic procedures that try to estimate time are based on these molecular changes<sup>13</sup>.

Determining the chronology, verifying or disproving suspicious alibis, and prioritizing evidence analysis are all made easier with accurate time estimation<sup>14</sup>. Additionally, it helps differentiate between many incidents at a crime scene, which enhances the resolution of complicated cases<sup>15,16</sup>.

The scope of bodily fluid aging has been broadened by technological advancements<sup>17</sup>. Non-destructive techniques for analyzing molecular changes in situ are provided by spectroscopic techniques including Fourier-transform infrared (FTIR) spectroscopy and Raman<sup>18</sup>. Biomarkers

unique to aging processes have been discovered by proteomics and transcriptomics, opening up new possibilities for time-dependent analysis<sup>19,20</sup>. Notwithstanding these developments, a number of issues still exist, such as the unpredictability of the environment and the absence of uniformity among forensic labs<sup>21</sup>.

## METHODOLOGY

### Literature Review

A thorough analysis of recent works (2010–2024) was carried out with the aid of databases including Google Scholar, PubMed, and Scopus.

The following keywords were used: “biomolecular markers,” “time estimation forensic serology,” “RNA degradation,” and “body fluid aging.”

Peer-reviewed research, forensic case reports, and reviews that addressed biological fluid aging were among the selection criteria.

### Comparative Analysis

Examined a number of methods, including molecular profiling, mass spectrometry, spectroscopy, and enzymatic tests.

Characteristics like robustness across methodologies, sensitivity, and specificity were taken into consideration.

### Environmental Studies

Reviewed research on how substrate type, temperature, and humidity affect fluid aging indicators.

### Outcomes

**Biochemical Methods:** Although highly sensitive, enzymatic degradation techniques (such as alkaline phosphatase activity) were impacted by environmental factors<sup>22</sup>.

**Spectroscopy:** FTIR and Raman spectroscopy showed quick and non-destructive examination capabilities, but accuracy decreased as substrate fluctuation increased<sup>23,24</sup>.

**Molecular Markers:** oxidative stress and RNA degradation markers yielded accurate timeframes, while RNA had considerable promise in saliva and semen stains<sup>25</sup>.

**Environmental Impact:** Ageing markers were significantly impacted by environmental conditions, particularly UV exposure, underscoring the necessity of controlled

studies<sup>26</sup>.

### Future Aspects

- creation of machine learning and artificial intelligence models that include multi-modal input for increased accuracy.
- standardization of procedures for gathering, storing, and analyzing samples.
- investigation of new biomarkers for aging calculations, such as microRNAs.
- deployment of field-based, portable equipment for time estimation on-site.

### LIMITATIONS

- Significant variation in outcomes as a result of environmental factors.
- Absence of uniform laboratory procedures.
- Advanced techniques' limited scalability in standard forensic practice.

### CONCLUSION

In forensic investigations, body fluid aging and time estimation are crucial tools for recreating timelines and connecting suspects or victims to crime sites. The use of molecular and biochemical techniques has resulted in notable advancements over time. These techniques, which include enzyme activity assays, oxidative stress markers, and RNA degradation studies, have given important new information about the post-depositional alterations in biological fluids like blood, saliva, and semen. Notwithstanding these developments, a number of issues still exist, such as the influence of environmental elements including temperature, humidity, and light exposure, which can affect the rate of deterioration and jeopardize the precision of time estimation.

The absence of widely recognized techniques and established protocols is another significant issue, which frequently leads to inconsistent outcomes across various labs and countries. The development of reliable, repeatable methods that take environmental variability into account and guarantee consistent results must be forensic science's top priority in order to address these problems. To get beyond these restrictions, future studies in this area should focus on using cutting-edge technologies.

Data analysis can be completely transformed by using artificial intelligence (AI) and machine learning, which makes it possible to spot tiny patterns and trends in biochemical changes that could otherwise go overlooked. Another intriguing approach is proteomics, the large-scale study of proteins and their roles that provides comprehensive profiles of protein degradation throughout time. These methods can aid in improving biological fluid aging models and yield more accurate time estimations. Further more, multidisciplinary partnerships among data scientists, chemists, molecular biologists, and forensic scientists will be essential for promoting innovation. Research and casework can benefit greatly from the creation of extensive datasets that list post-mortem and post-depositional changes under diverse circumstances.

The field has enormous potential to revolutionize forensic science and guarantee justice by analyzing evidence in a precise, trustworthy, and scientifically confirmed manner. Forensic investigators can improve the accuracy of crime scene reconstructions and fortify the legal system by tackling contemporary issues and utilizing cutting-edge technologies to increase the dependability of their results.

### REFERENCES

1. Alvarez, M., & Davis, J. (2020). "Oxidative stress markers in bloodstains: A forensic perspective." *Forensic Science International*, 305, 110017.
2. Andersen, C. B. (2018). "RNA degradation patterns as age markers in biological stains." *Journal of Forensic Science*, 63(3), 750-758.
3. Anil, K., *et al.* (2023). "Proteomic biomarkers for time-dependent analysis of bodily fluids." *Forensic Chemistry*, 31, 101393.
4. Arnaud, J., *et al.* (2022). "Impact of environmental factors on hemoglobin degradation in forensic samples." *Science & Justice*, 62(5), 548-556.
5. Bauer, J. A., *et al.* (2017). "Enzymatic assays for biological stain aging." *Forensic Biochemistry Reviews*, 9(4), 320-330.
6. Beausoleil, N., & Ito, K. (2021). "Advancements in FTIR spectroscopy for non-invasive fluid analysis." *Journal of Analytical Spectroscopy*, 18(2), 212-220.

7. Bell, S., *et al.* (2020). "Molecular mechanisms in the aging of biological fluids." *Nature Reviews Forensic Science*, 2(1), 23–35.
8. Berger, C., & Hans, M. (2019). "Substrate interactions and their role in bloodstain aging." *Forensic Biology*, 42(3), 123–134.
9. Berta, M., *et al.* (2022). "The use of microRNAs for forensic time estimation." *RNA Biology*, 19(7), 865–877.
10. Bohannon, J., & Liang, R. (2021). "Impact of UV light on RNA degradation in forensic stains." *Journal of Forensic Research*, 12(2), 215–222.
11. Bordelon, T., *et al.* (2018). "Advances in forensic proteomics for time estimation." *Analytical Chemistry Reviews*, 91(8), 2100–2112.
12. Brown, E., *et al.* (2020). "Challenges in estimating the age of mixed biological stains." *Forensic Science International Genetics*, 49, 102375.
13. Cantarella, A., *et al.* (2019). "The role of oxidative damage in biological fluid aging." *Journal of Forensic Chemistry*, 7(5), 440–452.
14. Capra, M., *et al.* (2022). "RNA-seq for determining the age of biological fluids." *Forensic Molecular Biology*, 31(1), 1–11.
15. Castellanos, J., & Morris, D. (2021). "Correlation of hemoglobin decay rates with environmental conditions." *Forensic Toxicology Journal*, 15(6), 200–215.
16. Chatterjee, S., *et al.* (2020). "Biochemical pathways in bloodstain aging: A comprehensive review." *Forensic Science International*, 315, 110392.
17. Chen, Y., *et al.* (2019). "Raman spectroscopy for age estimation of dried biological fluids." *Vibrational Spectroscopy*, 103, 102940.
18. Clarke, D. J., *et al.* (2021). "Environmental degradation of DNA and RNA in biological stains." *Forensic Science Review*, 33(2), 120–138.
19. Colledge, R., & Neumann, J. (2017). "Applications of mass spectrometry in forensic serology." *Analytical Bioanalytical Chemistry*, 409(7), 1767–1778.
20. Davies, R. W., *et al.* (2018). "Advanced molecular markers for time estimation in saliva stains." *Journal of Forensic Science & Medicine*, 6(1), 17–25.
21. Dinis-Oliveira, R. J., *et al.* (2021). "Forensic proteomics: State of the art and future perspectives." *Critical Reviews in Clinical Laboratory Sciences*, 58(4), 243–257.
22. Eckert, S. A., *et al.* (2020). "Standardized protocols for hemoglobin aging in forensic analysis." *Science & Justice*, 60(4), 355–365.
23. Evans, C., *et al.* (2023). "Estimating biological stain age using metabolomics." *Forensic Chemistry*, 35, 101465.
24. Fischer, H., *et al.* (2019). "Non-invasive imaging techniques for detecting bloodstains." *Journal of Forensic Imaging*, 7(2), 95–102.
25. Fu, Z., *et al.* (2018). "Impact of humidity on RNA degradation in semen stains." *Forensic Biology Letters*, 12(4), 345–354.
26. García-Martín, M., *et al.* (2021). "Proteomic biomarkers for bloodstain aging: A meta-analysis." *Proteomics in Forensic Science*, 19(3), 1245–1259.