

Impact on Creatinine Detection from Urine Stains of Different Fabric Surfaces for Forensic Identification using Jaffe's Method

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Abstract

The identification of urine stains at crime scenes plays a crucial role in forensic investigations, particularly in cases where other biological evidence is absent. This study evaluated the effect of different fabric surfaces on the persistence and detectability of creatinine in urine stains using Jaffe's Method. Five commonly encountered fabrics such as cotton, rayon, silk, polyester, and chiffon were selected for analysis. Equal quantities of urine samples were deposited on each fabric and allowed to dry under controlled environmental conditions. The persistence study was conducted for 40 days at fixed intervals to determine the detectability of creatinine over time. Jaffe's reaction was used for identification, where creatinine reacts with alkaline picric acid to produce an orange-red coloured complex. The findings revealed that creatinine detectability gradually decreased with time on all fabrics; however, the retention capacity varied significantly depending on the fabric type. Rayon showed the highest persistence with minimal fading even after 40 days, whereas polyester exhibited rapid loss of detectability. Cotton demonstrated moderate persistence, while silk and chiffon showed reduced retention with progressive fading after 20 days. The study concludes that both fabric type and aging period significantly influence creatinine detection in urine stains. These findings are important for forensic investigations involving aged biological stains and support the need for substrate-specific interpretation during urine stain analysis.

Keywords: Creatinine Detection, Urine Stains, Jaffe Reaction, Fabric Persistence, Forensic Identification, Cotton, Rayon, Polyester

1. Introduction

Urine is important biological evidence in forensic science, especially in cases where other biological fluids are absent. It contains metabolic waste products such as urea, uric acid, and creatinine, which can be used for forensic identification and toxicological analysis. Among these components, creatinine is considered a reliable biomarker because of its relative chemical stability and consistent presence in urine.[1,2]

The Jaffe Reaction sticks around in forensic and clinical labs for one big reason: it's cheap and fast. All you need is picric acid and a simple alkaline solution like sodium hydroxide stuff that's easy to find and it's cheaper. This means even labs with limited budgets can use it. we don't need fancy machines or a ton of training either; basic lab gear gets the job done, and sometimes we can even spot the colour change with our own eyes for a quick, rough identification. Speed is another plus. Mix the reagents with our

sample, and within minutes, we'll see a bright orange-red colour pop up. That kind of turnaround matters, especially in forensic work, where time can be everything. If we need actual numbers, just pop the sample into a spectrophotometer no complicated steps needed. Sure, there are possible interferences, but we can't beat the method's simplicity, fast results, and low cost. That's why the Jaffe reaction is still the go-to choice for creatinine detection, especially when we're dealing with high-volume screenings or working out in the field.

The Jaffe Reaction is one of the most commonly used methods for creatinine detection. In this reaction, creatinine reacts with alkaline picric acid to produce an orange-red coloured complex known as creatinine picrate. The intensity of the colour indicates the presence and relative concentration of creatinine.

In forensic investigations, urine stains may be deposited on different textile surfaces such as cotton, silk, rayon, polyester, and chiffon. The physical and chemical properties of these fabrics influence the absorption, retention, and degradation of urine stains. Natural fabrics generally absorb fluids more efficiently, while synthetic fabrics exhibit poor retention due to hydrophobic characteristics.

This study focuses on evaluating the persistence of creatinine in urine stains deposited on various fabrics over a period of 40 days using Jaffe's Method. The study aims to determine how different textile surfaces influence the detectability and forensic reliability of urine stains.

2. Aim of the Study

To evaluate the effect of different fabric surfaces on the detection and persistence of creatinine in urine stains using Jaffe's Method for forensic identification.

3. Literature review

Heinsvig et al. (2024) conducted a forensic study on urine analysis in clinical forensic cases involving victims and perpetrators. The study highlighted the importance of urine as biological evidence and emphasized the role of chemical biomarkers such as creatinine in forensic toxicology and biological identification. The researchers concluded that urine analysis remains highly useful in cases where other biological evidence is absent.

Delanghe and Speeckaert (2011) reviewed the significance of creatinine determination using the Jaffe reaction. Their study explained the chemical principles behind the formation of the orange-red creatinine-picric acid complex and discussed various interfering factors affecting the reliability of creatinine estimation. The study confirmed that the Jaffe method continues to be widely accepted due to its simplicity and cost-effectiveness.

Hager et al. (2018) investigated forensic identification of urine stains on cotton and polyester fabrics using Raman spectroscopy. The findings demonstrated that fabric composition significantly affects the retention and detection of urinary residues. Cotton retained urinary compounds for longer durations, while polyester showed rapid degradation due to poor absorbency.

Chromý et al. (2008) studied creatinine detection using the Jaffe method and focused on reducing matrix interference during analysis. Their research highlighted the importance of accurate colour interpretation in creatinine analysis and suggested that substrate characteristics can influence colour development and reaction intensity.

Bharti Jain et al. (2020) conducted a persistence study on biological stains deposited on different fabrics. Although the study focused on semen stains, the results demonstrated that natural fabrics preserve

biological materials more effectively than synthetic fabrics. The study provided important forensic insight into the role of textile composition in stain persistence.

Syal et al. (2013) analysed various interferences associated with creatinine estimation and compared enzymatic and Jaffe-based methods. The researchers concluded that environmental conditions and sample contamination may influence colour intensity and detection reliability over time.

4. Objectives of the Study

- To evaluate the persistence of creatinine in urine stains on different fabric surfaces over a defined time interval.
- To analyse changes in colour intensity of the creatinine-picric acid complex over time.
- To compare the retention capacity of natural and synthetic fabrics.
- To determine the longest duration for which creatinine remains detectable on each fabric.
- To assess the forensic reliability of aged urine stains using Jaffe's Method.

5. Research Gap

Although several studies have explored creatinine detection methods and urine analysis, limited research has been conducted on the influence of different textile substrates on creatinine persistence in dried urine stains. Existing studies primarily focus on clinical applications or controlled laboratory conditions rather than real forensic scenarios involving various fabrics. There is insufficient comparative data regarding the effect of natural and synthetic fabrics on urine stain retention, degradation, and colour intensity during the Jaffe reaction.

Furthermore, factors such as stain aging, environmental exposure, and substrate interference remain inadequately studied. Therefore, this study addresses the lack of systematic experimental analysis regarding the persistence of creatinine on commonly encountered fabrics used in forensic investigations.

6. Materials and Methods

6.1 Research Design

- Type of Study: Experimental Longitudinal Study
- Nature of Study: Time-dependent persistence analysis
- Duration: 40 days

6.2 Fabric Samples

The following fabrics were selected for analysis:

- Cotton
- Rayon
- Silk
- Polyester
- Chiffon

6.3 Chemicals Used

- Picric Acid
- Sodium Hydroxide (NaOH)
- Distilled Water

6.4 Sample Preparation

Equal-sized fabric samples measuring approximately 1.5×1.5 cm were prepared. Fresh urine samples

from healthy volunteers were collected and deposited uniformly onto each fabric surface. The stained fabrics were dried under controlled room conditions.

6.5 Detection Procedure

Jaffe’s Method was performed by spraying:

1. Picric acid solution
2. Sodium hydroxide solution

The reaction was allowed to proceed for 5–10 minutes. The appearance of orange-red colour indicated the presence of creatinine.

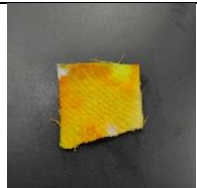

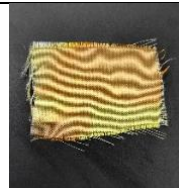
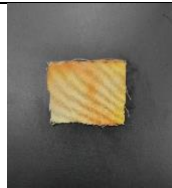
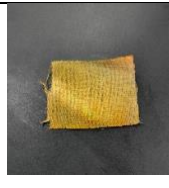
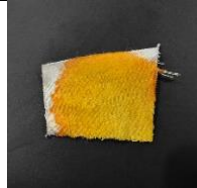
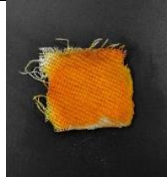

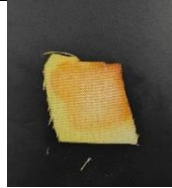




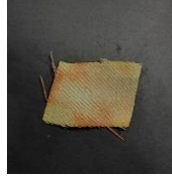

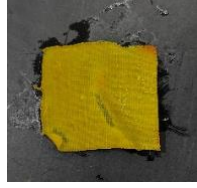
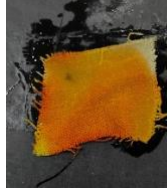

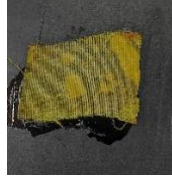

6.6 Grading System

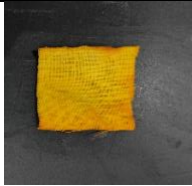


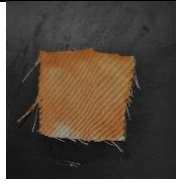

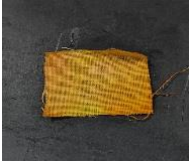





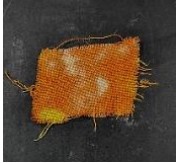
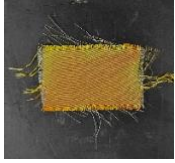
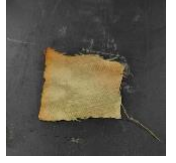
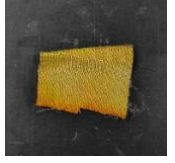
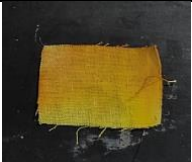


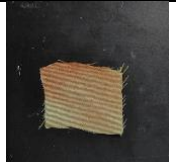


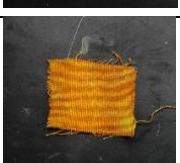




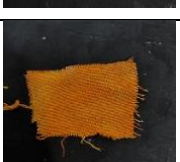


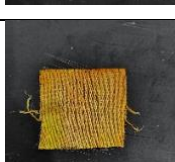
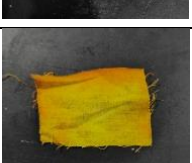
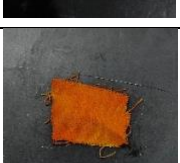
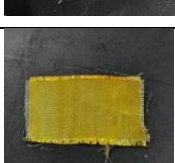
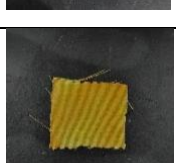
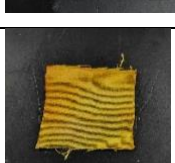
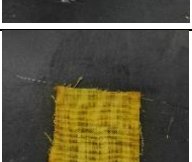
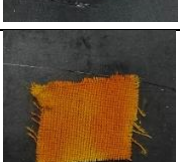


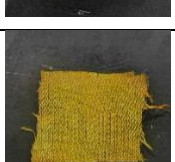
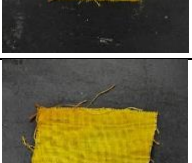
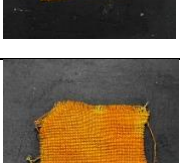
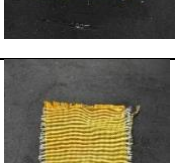
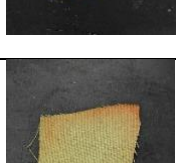
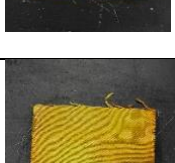
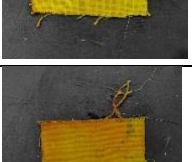
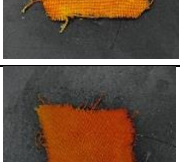
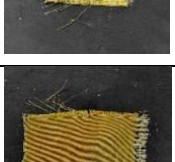
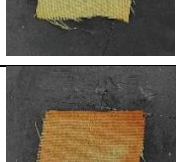
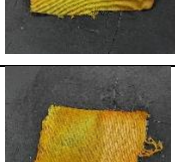
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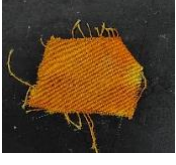

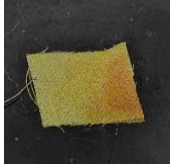



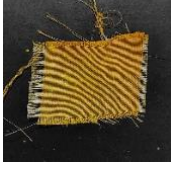

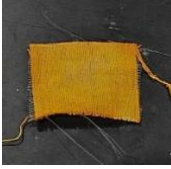



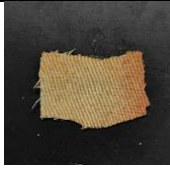
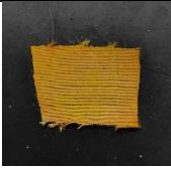


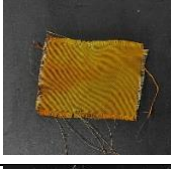
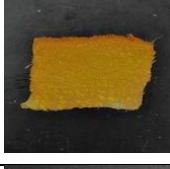
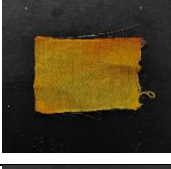
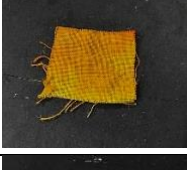
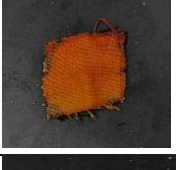

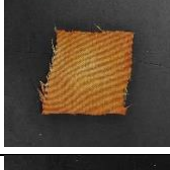
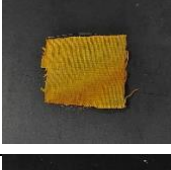

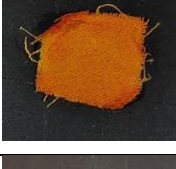
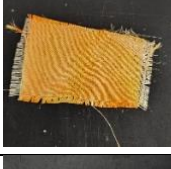

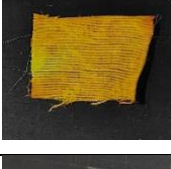



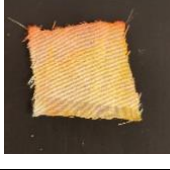




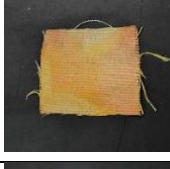
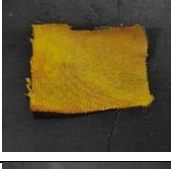
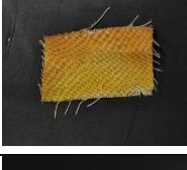







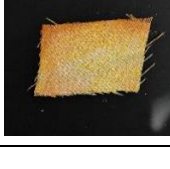

Grade	Observation
Grade I	Full colour intensity
Grade II	Minimal reduction in colour
Grade III	Half reduction in colour
Grade IV	Maximum colour reduction


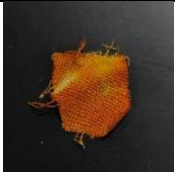

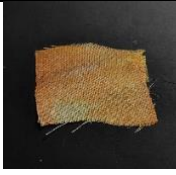


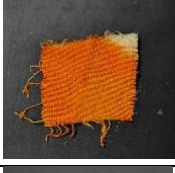
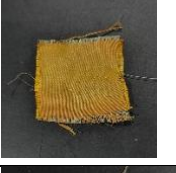
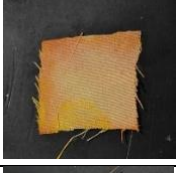

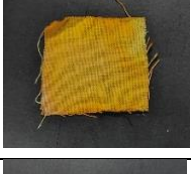
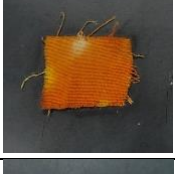
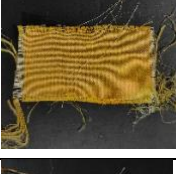
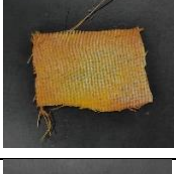
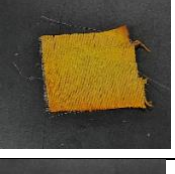
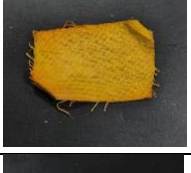
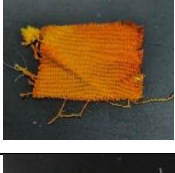
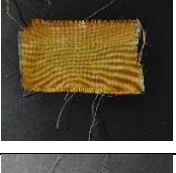
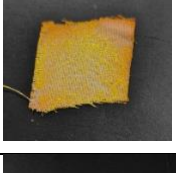
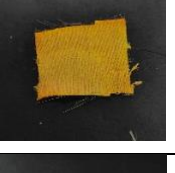
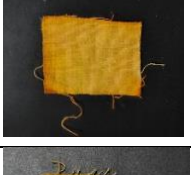
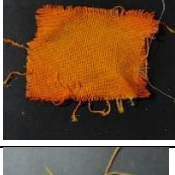
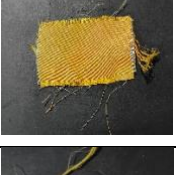
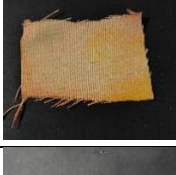
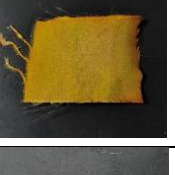

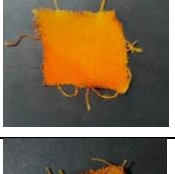

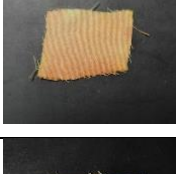
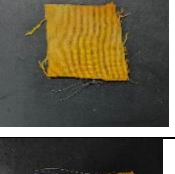

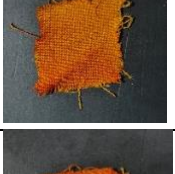
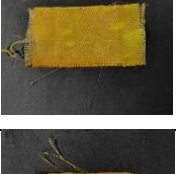
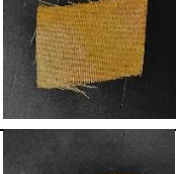
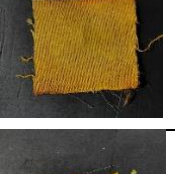
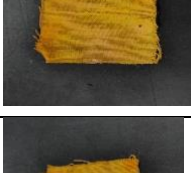
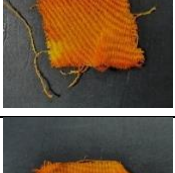
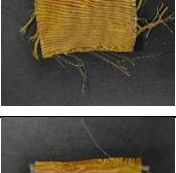
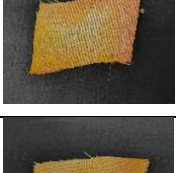
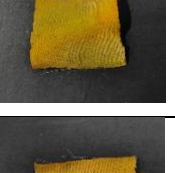
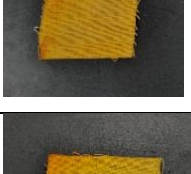
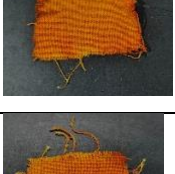
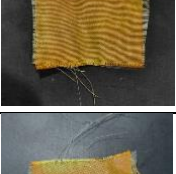
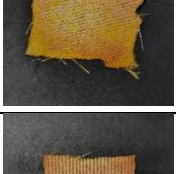
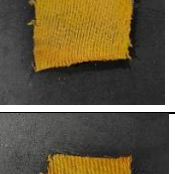

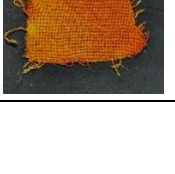

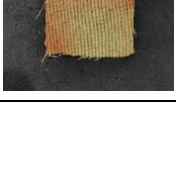

7. Results and Discussion



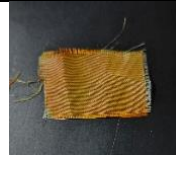

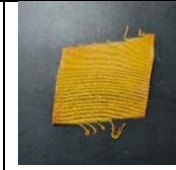


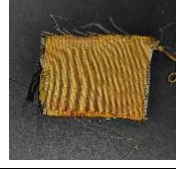
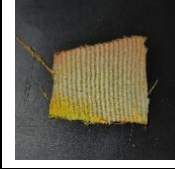
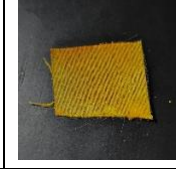

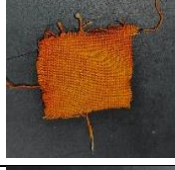
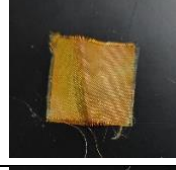
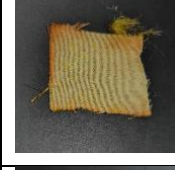
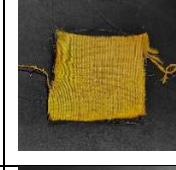








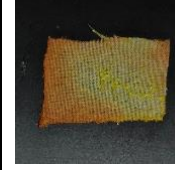
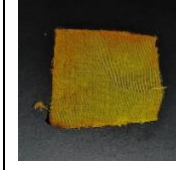

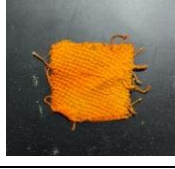
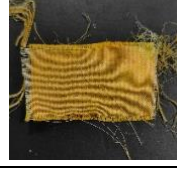
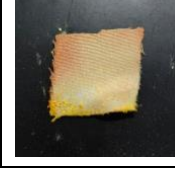
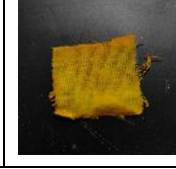
The persistence of creatinine in urine stains on different fabrics was analysed for 40 days using the Jaffe Reaction. All samples initially showed positive colour development, confirming the presence of creatinine.

	S1 (Cotton)	S2 (Rayon)	S3 (Silk)	S4 (Polyester)	S5 (Chiffon)
Day 1					
Day 2					
Day 3					
Day 4					

Day 5					
Day 6					
Day 7					
Day 8					
Day 9					
Day 10					
Day 11					
Day 12					
Day 13					
Day 14					

Day 15					
Day 16					
Day 17					
Day 18					
Day 19					
Day 20					
Day 21					
Day 22					
Day 23					
Day 24					

Day 25					
Day 26					
Day 27					
Day 28					
Day 29					
Day 30					
Day 31					
Day 32					
Day 33					
Day 34					

Day 35					
Day 36					
Day 37					
Day 38					
Day 39					
Day 40					

Grading and Data Analysis:

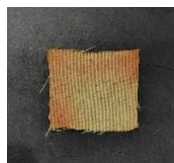
Grade 1 – Full colour intensity



Grade 2 – Minimal reduction in colour



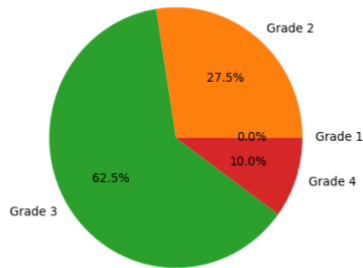
Grade 3 – Half reduction in colour



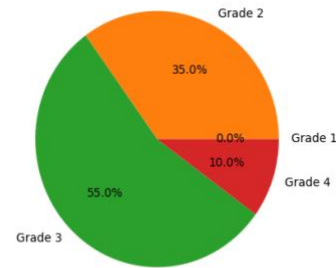
Grade 4 – Maximum colour reduction



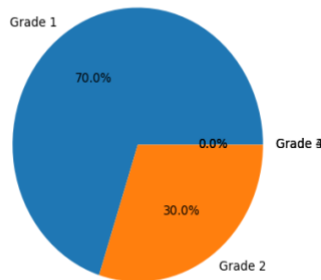
Cotton [S1] - Persistency Distribution (40 Days)



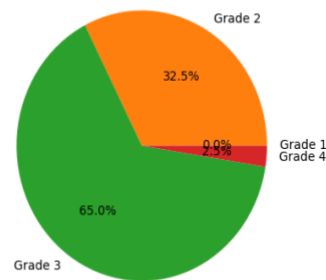
Silk [S3] - Persistency Distribution (40 Days)



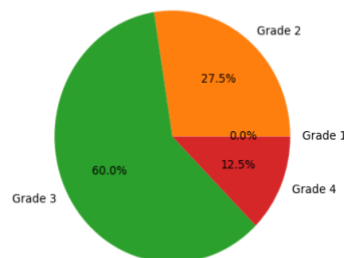
Rayon [S2] - Persistency Distribution (40 Days)



Polyester [S4] - Persistency Distribution (40 Days)



Chiffon [S5] - Persistency Distribution (40 Days)



7.1 Cotton

Cotton exhibited strong absorbency and showed intense colour formation during the early days of the study. Fading became noticeable after 10 days and significant reduction occurred after 20 days. However, faint positive reactions persisted until the 40th day.

7.2 Rayon

Rayon demonstrated the highest stability among all tested fabrics. Minimal reduction in colour intensity

was observed throughout the study period. Detectable creatinine reactions remained visible even on the 40th day, indicating superior retention properties.

7.3 Silk

Silk showed moderate colour intensity initially. Gradual fading was observed after 10 days, with more significant reduction after 20 days. Only weak positive reactions remained at the end of the persistence study.

7.4 Polyester

Polyester displayed poor absorbency and weak retention of urine stains. Rapid fading was observed after 10 days, and detection became very weak after 20 days. Polyester showed the least reliable persistence among all fabrics.

7.5 Chiffon

Chiffon demonstrated rapid spreading and evaporation of urine stains due to its lightweight structure. The colour intensity reduced significantly over time, with only minimal detectable reactions remaining on the 40th day.

7.6 Comparative Analysis

The overall persistence and retention capacity of fabrics followed the order:

Rayon > Cotton > Silk > Chiffon > Polyester

The grading system and observational findings confirmed that fabric composition significantly influences creatinine detectability. Natural and semi-synthetic fabrics retained urine stains more effectively than synthetic fabrics.

8. Conclusion

The present study confirms that the Jaffe Reaction is an effective method for detecting creatinine in urine stains; however, the reliability of detection is significantly influenced by the fabric substrate and duration of stain aging. Rayon exhibited the highest persistence and retention of creatinine throughout the 40-day study period, while polyester demonstrated the poorest retention due to its hydrophobic nature.

The findings indicate that absorbent fabrics preserve urinary components for longer durations, whereas synthetic fabrics promote rapid evaporation and degradation. Significant fading was observed after 10 days in most fabrics, and drastic reduction in detectability occurred after 20 days.

Therefore, forensic investigators must consider fabric type while interpreting urine stain evidence. The study highlights the importance of substrate-specific analysis in forensic examinations involving aged biological stains.

9. Significance of the Study

This study contributes to forensic science by providing comparative information regarding creatinine persistence on different textile surfaces. The findings can improve forensic interpretation of urine stains, reduce false-negative results, and support more accurate crime scene reconstruction.

10. Limitations of the Study

- Environmental conditions such as sunlight and humidity were not extensively evaluated.
- Individual variations in urine composition were not considered.
- Interference from detergents and fabric dyes was not fully analysed.

11. Acknowledgement

We express our sincere gratitude to Dr. M.G.R Educational and Research Institute for providing the necessary facilities, infrastructure, and academic support for the successful completion of this research work.

We are deeply thankful to the Department of Criminology and Forensic Science for their constant encouragement, guidance, and technical assistance throughout the study.

Special thanks are extended to Ms. Akshara M P, Assistant Professor, Department of Criminology and Forensic Science, Dr. M.G.R Educational and Research Institute, for her valuable supervision, continuous support, constructive suggestions, and encouragement during every stage of the research process.

We also express heartfelt gratitude to all faculty members, friends, and well-wishers who directly or indirectly contributed to the successful completion of the study.

12. References

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