

Influence of Fabric Type, Blood Drop Volume, and Environmental Conditions on Drying Behaviour and Morphological Characteristics of Bloodstains on Textile Substrates

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Abstract:

Bloodstain drying behaviour on textiles plays a crucial role in forensic interpretation, yet systematic data on how fabric structure, environmental temperature, and blood volume jointly influence stain development remain limited. This study examined the drying times and morphological characteristics of blood droplets deposited on four common plain woven textile substrates: cotton, nylon, silk, and polyester. Three blood volumes (10 μ L, 15 μ L, 18 μ L) were deposited using a micropipette. Experiments were conducted under three controlled environmental conditions that represented typical real-world scenarios (room temperature, 50 °C oven, 3.5–6 °C refrigeration). Each condition was performed in triplicate. Fabrics chosen were plain-woven patterns of Nylon, Polyester, Cotton and Silk (3 × 3 cm) which were suspended over open glass vials to eliminate second-surface interaction, ensuring that stain formation reflected true fabric–blood behaviour.

Distinct substrate-specific morphological patterns were observed. Nylon exhibited minimal absorption, forming raised, dome-like stains. Cotton absorbed rapidly and deeply, producing fully saturated stains with later formation of a central darkened loop at warmer temperatures. Silk initially allowed lateral spread before constricting into a pooled centre depression that produced a characteristic “central loop” morphology. Polyester, despite being non-absorbent, showed partial absorption under these conditions, initially forming an elliptical translucent halo followed by a defined circular centre. Temperature significantly influenced drying time: stains dried fastest in the oven and slowest in the refrigerator, though cooler conditions produced brighter, more vivid stain colouration.

These findings contribute baseline data for interpreting bloodstains on textiles where fabric type, temperature, and volume are unknown variables. Understanding these interactions strengthens the reliability of forensic bloodstain pattern analysis in crime scene reconstruction.

Keywords: Bloodstain drying, textile substrates, Stain morphology, Temperature effects, Substrate absorption, Forensic bloodstain analysis

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Introduction

Bloodstain pattern analysis (BPA) is widely used in forensic reconstruction to understand events surrounding violent incidents. Accurate interpretation relies heavily on recognising how blood behaves once deposited, including the processes of spreading, absorption, and evaporation. Although several studies have examined drying dynamics on smooth, nonporous surfaces, the behaviour of blood on woven textiles remains significantly underexplored despite being one of the most common substrates encountered in casework. (O Peschel, 2011) (Tom Bevel, 2008)

Existing literature shows that blood undergoes a predictable series of physical transitions during drying, beginning with coagulation and progressing through rim formation, gelation, and central desiccation. (Laan, 2016) These stages are largely reproducible under controlled laboratory conditions, but the timing and morphology depend heavily on substrate properties and environmental conditions. Studies of blood pools and droplets on rigid hydrophilic and hydrophobic surfaces have demonstrated clear relationships between substrate wettability, evaporation dynamics, and fracture patterns. (Iqbal, 2020) However, findings from non-porous surfaces do not fully translate to textiles, where capillary flow, fibre morphology, and fabric permeability alter the way blood spreads, absorbs, and dries.

Research on woven fabrics indicates that stain shape is influenced by capillary transport within yarns and the interaction between the droplet and the fibre network (Agrawal, 2017). Absorbent fabrics such as cotton tend to pull blood into the substrate, allowing imbibition to dominate stain formation. In contrast, non-absorbent or low-absorbent materials produce surface-dominated pools with limited penetration. (H.F. Miles, 2014) This distinction can distort directional angles, change stain boundaries, and obscure essential features required for reconstruction. Yet, the majority of published textile-focused BPA research is limited to unidirectional drip stains and does not systematically compare fabric behaviours across environmental temperatures. (Jiaying Wu M.S., 2018)

Temperature is another factor with significant forensic implications. Prior work shows that elevated temperatures accelerate drying rates, while cooler environments prolong moisture retention and slow morphological transitions. (van Oorschot, 2014) Temperature also influences DNA persistence and transferability, reinforcing the need to understand

drying dynamics in realistic ranges. However, very few studies evaluate temperature effects specifically on fabric-borne droplets, where the combination of substrate and thermal environment may produce unique morphological outcomes.

This gap poses a practical challenge for forensic analysts: the morphology and drying stage of a bloodstain on clothing may hold valuable temporal information, but without robust reference patterns, interpretation remains uncertain. As a result, there is a need for empirical data that link fabric characteristics, droplet volume, and environmental conditions to measurable changes in drying behaviour and stain appearance.

The present study contributes to this area by examining how three blood volumes dry on four commonly encountered textile substrates: cotton, silk, polyester, and nylon under controlled room-temperature, warm (oven), and cold (refrigerated) environments. By integrating fabric behaviour, temperature variation, and controlled droplet volumes, this study contributes new data to an area of BPA that remains under-represented in the literature. Its findings aim to support forensic examiners by improving expectations of how stains form, evolve, and appear on different textiles. Ultimately, the study provides experimentally grounded insight that may help refine crime-scene interpretation when bloodstains on clothing are involved.

Aim

This study aimed to determine how fabric type, droplet volume, and environmental temperature influence the drying times and morphological characteristics of bloodstains on textiles.

Research Questions

1. How do drying times vary across cotton, silk, polyester, and nylon under different temperatures?
2. What morphological features emerge during drying on each fabric?
3. How does blood volume affect spread, absorption behaviour, and final stain appearance?
4. How can these observations support forensic interpretation of bloodstains on clothing?

3. MATERIALS & METHODS

3.1. Blood Source and Preparation

Defibrinated horse blood (TCS Biosciences, UK) was used throughout the experiment. Two conditions of blood were prepared: *fresh blood*, which had been refrigerated for approximately one week, and *aged blood*, stored for two to four weeks under the same conditions. All samples were equilibrated to room temperature before deposition to ensure consistency in viscosity and flow behaviour.

3.2. Fabric Substrate

Four commonly encountered clothing fabrics (Plain woven) were selected for analysis: **cotton**, **silk**, **polyester**, and **nylon**. Each fabric was cut into standardised 3 × 3 cm squares. To eliminate second-surface interference, all fabric samples were placed flat over the open end of a sterile glass vial, allowing the droplet to interact solely with the textile surface without contact pressure or underlying absorption (Laan, 2016).

3.3 Environmental Conditions

Drying experiments were conducted under three controlled environments designed to reproduce realistic climatic scenarios:

- **Room temperature:** 18–22 °C
- **Warm/oven condition:** 50 °C
- **Refrigerated condition:** 3.5–6 °C

All environments were stabilised for at least 15 minutes before sample placement to ensure uniform thermal exposure.

3.4 Blood Deposition Protocol

Blood droplets of **10 µL**, **15 µL**, and **18 µL** were deposited onto the centre of each fabric square using a calibrated micropipette. Droplets were released vertically from a minimal height to avoid impact dynamics and ensure uniform deposition across trials.

3.5 Drying Time Measurement

Drying time was recorded manually using a digital timer placed adjacent to the experimental setup. Timing began immediately after droplet deposition. Samples were periodically checked, and drying was

assessed using a **pin-test**: a sterile pin was lightly touched to the stain surface.

Drying was defined as the point at which the pin showed **no visible transfer** and caused **minimal to no surface disruption**. This operational definition ensured consistency across all substrates and conditions.

3.6 Microscopic Imaging and Morphology Assessment

Morphological characteristics of the dried stains were examined using a Keyence VHX-2000 digital microscope at 20× magnification. High-resolution images were captured both before and after blood deposition to document substrate structure, weave pattern, and subsequent stain formation.

Attempts were made to evaluate absorption depth and internal stain architecture; however, limitations of the equipment prevented precise quantitative measurement. Depth-related morphological cues (e.g., central thickening, surface pooling, peripheral thinning) were described only qualitatively.

3.7 Data Handling and Documentation

All measurements, times, and observations were recorded immediately during experimentation. Images and Keyence digital microscopy recordings were archived systematically for later analysis.

4. Results

4.1 Drying Time Across Environmental Conditions

Drying times varied substantially between polyester and nylon under all three temperatures (Table 1). Polyester consistently exhibited the shortest drying durations, whereas nylon required significantly longer periods, often exceeding the allocated observation window at low temperatures.

Polyester

- At room temperature (19–22 °C), drying occurred within **1–3 minutes** across all volumes.
- In the oven (50 °C), drying accelerated further, with the smallest volume (10 µL) drying in **1 minute 16 seconds**.

- In refrigerated conditions (3.5–6 °C), drying slowed but remained within measurable limits, ranging between **8–20 minutes** depending on volume.

Nylon

- At room temperature, drying ranged from **1 hour 44 minutes to over 2 hours**, showing markedly prolonged evaporation compared with polyester.
- In the oven, drying was faster but still significantly delayed (approx. **35–39 minutes**).
- In the refrigerator, all volumes exceeded the experimental limit and did not fully dry within the observation period.

These data establish polyester as the most rapid-drying substrate tested, and nylon as the slowest across temperatures.

Temperature	Polyester	Nylon
Room Temperature 19- 22°C	10µL = 00:01:44	10µL = 01:44:20
	15µL= 00:02:47	15µL= 02:08:43
	18µL= 00:03:01	18µL= 02:12:26
Oven 50 °C	10µL = 00:01:16	10µL = 00:35:29
	15µL= 00:01:34	15µL=00:36:13
	18µL= 00:02:44	18µL= 00:39:19

Fridge 3.5- 6 °C	10µL = 00:07:59	10, 15 & 18µL = exceeded the given time.
	15µL= 00:15:24	
	18µL= 00:20:11	

Table 1: Mean drying times (hr:min:s) for 10, 15, and 18 µL blood droplets deposited on polyester and nylon across three temperature conditions.

4.2. Morphological Characteristics of Dried Stains

The morphology of dried stains showed distinct substrate-dependent patterns (Figure 1).

Polyester

- Stains displayed **an initial translucent elliptical spread**, followed by the formation of a **more concentrated circular centre** as drying progressed.
- Partial absorption occurred despite polyester being non-absorbent by nature, likely due to the absence of a second surface beneath the fabric during testing (glass vial support).
- Final morphology showed **smooth perimeters and uniform tone**, with little textural variation.

Nylon

- Blood droplets remained **pooled on the fabric surface**, forming **thick, dome-shaped, glossy deposits** with negligible absorption into fibres.
- A cross-sectional Keyence analysis confirmed **minimal absorption**, with the droplet sitting entirely above the textile structure.
- Morphology was dominated by **surface retention** rather than diffusion, producing highly defined edges.

- Cracking patterns appeared earlier under oven conditions, while refrigerated droplets showed delayed but progressive cracking.

Cotton

- Droplets were **fully absorbed**, creating diffuse, irregular stains with darker perimeters.
- Absorption into the woven structure produced characteristic **inner ring formation** at room and warm temperatures, although this ring was **absent in refrigerated samples**.

Silk

- Showed initial lateral spread before blood accumulated centrally, forming the characteristic inner “blood loop.”
- Absorption visibly altered the silk fibres, creating a slightly crinkled appearance.

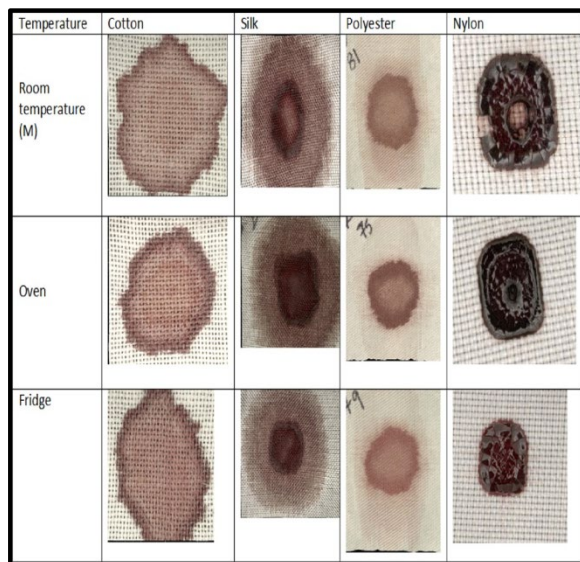


Figure 1: Images display substrate-specific differences in colour intensity, spreading behaviour, and edge formation of blood drops on different fabrics under varying environmental conditions

4.3 Depth and Surface-Topography Analysis

Keyence VHX-2000 digital microscopy (20× magnification) provided qualitative surface topography and depth information for each substrate (Figure 2).

- Cotton** showed widespread depth variation consistent with complete absorption and fibre saturation.
- Silk** depth mapping revealed moderate absorption with a clear central depression consistent with the pooled blood.
- Polyester** showed shallow penetration with a mostly uniform profile.
- Nylon** displayed a **pronounced elevated dome**, confirming that the droplet remained on the surface with almost no infiltration.

Exact depth values could not be quantified due to limitations of the instrument; therefore, depth observations are qualitative.

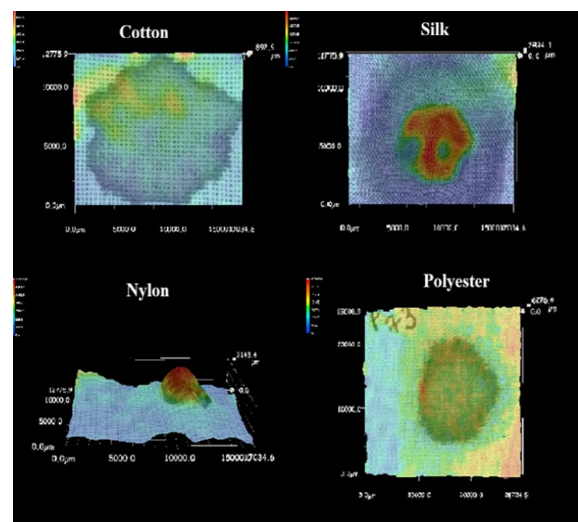


Figure 2: Keyence depth-profile images showing relative absorption characteristics of cotton, silk, polyester, and nylon.

4.4 Temperature Influence on Stain Appearance

Across all fabrics, temperature produced noticeable differences in visual characteristics:

- Refrigerated stains** appeared **brighter** in colour, retaining more visible red tones and a glossy finish.
- Oven-dried stains** were **darker**, with increased central density and sharper boundaries.

- **Room-temperature stains** fell between these extremes, showing moderate spread and colour intensity.

These temperature-dependent changes were consistent across replicates and visible both to the naked eye (Figure 1) and under Keyence microscopy (Figure 2)

4.5 Volume Effects on Drying and Morphology

Blood volume influenced both the duration of drying and the stain size across all fabrics:

- Larger droplets (18 μL) produced **broader stains** and required longer drying times than smaller droplets (10 μL).
- Morphology remained substrate-driven, but intensified it; for example, larger volumes increased central pooling on silk and exaggerated dome formation on nylon, but did not alter the fundamental morphological pattern determined by the substrate.

5. Discussion

This study examined how fabric type, droplet volume, and environmental temperature influenced the drying behaviour and final morphology of bloodstains. Across all conditions, polyester consistently produced the shortest drying times, whereas nylon showed markedly prolonged drying, particularly under low temperatures. These findings reflect fundamental differences in textile structure and liquid–substrate interactions, providing valuable insights for the forensic interpretation of bloodstains encountered on clothing.

5.1 Substrate-Dependent Drying Behaviour

A clear hierarchy in drying rate was observed among the four fabrics. Polyester consistently dried the fastest across all temperature conditions, whereas nylon exhibited the slowest drying, with droplets remaining undried within the allotted observation period in refrigerated conditions. Despite Polyester being a non-absorbent synthetic fibre, it permitted partial settling of the droplet within the inter-fibre gaps when no second surface was present, resulting in a thin, elliptical film that transitioned quickly into a defined central deposit. In contrast, nylon retained the droplet almost entirely on the surface, forming a raised, dome-shaped stain with negligible fibre penetration. This surface pooling substantially prolonged drying times.

Cotton and silk demonstrated distinct behaviours that contributed equally to the understanding of textile–blood interactions. Cotton fully absorbed the droplet, producing diffuse, irregular stains that expanded rapidly through the fibre network (Wang, 2025). Silk displayed a characteristic two-phase behaviour: an initial lateral spread, followed by blood migration back toward the centre as drying progressed, creating a well-defined inner loop unique to its structure. These substrate-specific differences highlight the combined role of fibre composition, weave structure, and capillarity in shaping both drying dynamics and resulting stain morphology (Agrawal, 2017) (H.F. Miles, 2014).

5.2 Effect of Environmental Temperature on Drying and Stain Appearance

Temperature exerted a clear influence on both drying behaviour and the visual characteristics of the resulting stains across all four fabrics (Figure 1). Higher thermal conditions consistently shortened drying times, while colder environments prolonged the process and altered the final appearance of the stains (van Oorschot, 2014).

Under oven conditions (50 °C), all fabrics demonstrated accelerated drying. Polyester and cotton dried particularly quickly due to efficient heat transfer through their fibre structures, whereas silk showed rapid solidification of the centrally pooled blood, producing a well-defined, dense core. Nylon, although inherently slow to dry, exhibited an earlier onset of peripheral cracking at this temperature, indicating accelerated surface desiccation despite minimal fibre absorption.

At room temperature (19–22 °C), drying proceeded at an intermediate rate for all substrates. Stains formed with characteristic morphology for each fabric, but without the pronounced colour shifts or accelerated structural changes observed under heated conditions.

In refrigerated conditions (3.5–6 °C), drying was markedly slower, and stains retained a noticeably brighter red colouration. This effect was visible across all fabrics and likely reflects reduced haemoglobin oxidation at lower temperatures. Morphological features were also temperature-dependent: cotton produced its typical diffuse absorption pattern but did not exhibit the central inner ring seen at higher temperatures; silk showed lateral spread with delayed central pooling, and nylon demonstrated very slow desiccation with late-stage surface cracking compared to more rapid fragmentation in the oven.

Overall, the temperature-dependent differences in colour intensity, structural definition, and rate of fluid solidification observed in this study align with controlled-environment research demonstrating the sensitivity of bloodstain appearance to thermal conditions. These effects, combined with substrate-specific interactions, underscore the importance of considering both temperature and textile properties when interpreting scene-based bloodstains.

5.3. Volume Effects on Stain Formation

Blood volume primarily influenced the *scale* of the stain and the total drying duration, while the fundamental behaviour of each substrate remained unchanged. Larger droplets (18 μL) consistently produced wider stains and required proportionally longer drying times across all environmental conditions. However, volume did not alter the characteristic absorption, pooling, or surface-retention behaviours that were specific to each fabric. Instead, it amplified those existing patterns, demonstrating that substrate properties rather than droplet size were the dominant factors governing drying dynamics and resultant stain characteristics.

5.4 Depth-Related Characteristics from Keyence Microscopy

The Keyence VHX-2000 microscopic analysis provided additional insight into the three-dimensional characteristics of stain formation. Although precise depth measurements could not be quantified due to instrument limitations, qualitative height mapping confirmed the substrate-dependent interactions observed visually.

The cross-sectional imaging clearly demonstrated that nylon absorbed negligible blood. The droplet remained raised above the fibre surface, producing a steep height gradient that corresponds with the dome-shaped morphology noted in the results. Polyester showed moderate subsurface penetration into the fibre gaps, producing a shallower elevation profile compared to nylon. Cotton displayed the most uniform depth distribution, supporting complete absorption and minimal height variation across the stain. Silk exhibited a distinct raised central region consistent with the inner pooling phenomenon, reflecting both moderate absorption and structural tightening of the fibres around the blood droplet.

These depth-related observations further reinforce the conclusion that fibre composition and weave structure are primary determinants of stain morphology. Figure 2 illustrates these height maps,

supporting the visual and quantitative observations described in the results.

5.5 Morphological Outcomes Across Fabrics

Distinct morphological patterns were observed across all four fabrics, reflecting differences in fibre structure, absorbency, and capillary behaviour. These characteristics were documented through direct visual assessment and verified using the Keyence VHX-2000 microscope at 20 $\times$ magnification (Figure 2), which provided high-resolution imaging of stain boundaries and limited cross-sectional depth profiles.

Polyester presented a two-stage morphology, beginning with a translucent, elliptical spread followed by the development of a more concentrated circular centre as drying progressed. Partial penetration into the inter-fibre gaps produced smooth edges and a uniform tonal appearance. These features remained consistent across temperatures, although oven-dried samples showed darker central regions due to accelerated desiccation.

Nylon produced highly distinctive dome-shaped stains, characteristic of its minimal absorbency. Keyence cross-sectional imaging confirmed that blood remained largely on the surface with negligible penetration into the fibre network. Dome height increased with droplet volume, and surface tension effects resulted in sharply defined borders. Cooler conditions delayed surface consolidation and maintained a bright red appearance, whereas oven conditions produced earlier darkening.

Cotton generated fully absorbed, diffuse stains with irregular outlines and darker edges indicating complete wicking into the fibre structure. Microscope imaging showed the absence of a raised surface deposit and instead a homogenised distribution within the fabric matrix. Under refrigerated conditions, the typical central ring did not form, reflecting slower clotting behaviour.

Silk displayed a characteristic progression in which early lateral spread was followed by central accumulation, producing the distinctive inner “blood loop” after drying. The fabric’s sensitivity to moisture caused subtle surface crinkling, which influenced final stain geometry. Higher temperatures intensified central pooling and produced more compact ring structures, whereas refrigerated samples maintained brighter red tones but exhibited reduced definition of the loop.

Collectively, the morphological outcomes demonstrate that each textile exhibits a unique interaction profile with deposited blood. These differences highlight the importance of recognising substrate-specific characteristics when interpreting stains on garments or household fabrics, particularly in reconstructions involving timing, movement, or thermal exposure.

5.6 Comparison with Existing Literature

The results align with and extend existing BPA research (Laan, 2016) characterised drying stages of pooled blood on non-porous surfaces, noting predictable, repeatable patterns; the present findings echo this reproducibility across fabrics under controlled conditions (van Oorschot, 2014), demonstrating that temperature dramatically accelerates blood drying, mirroring the strong thermal influence observed here. Studies on woven fabrics by (Agrawal, 2017) have shown that capillary transport distorts stain geometry, consistent with cotton's extensive absorption and the complexity of interpreting stains on porous substrates (Samira Shiri, 2019). This study highlights how surface properties can drastically alter stain shape, and the pronounced differences between nylon, polyester, and silk in this study similarly reflect substrate sensitivity.

Although the present study focuses solely on drying and morphology, the findings provide foundational insight into substrate-dependent behaviours that can inform broader interpretation frameworks in forensic practice.

5.7 Forensic Interpretation and Relevance

The findings demonstrate that bloodstains formed on textiles cannot be interpreted solely through principles established from smooth or nonporous surfaces (Koray Büyükatık MD, 2025). The type of fabric directly influenced drying speed, stain dimensions, edge formation, and colour appearance. Environmental temperature and droplet volume further modified these outcomes, meaning that identical droplets produced markedly different stain characteristics depending on substrate and climate.

These observations highlight several risks for casework interpretation. Rapid drying on polyester may resemble stains that are older than they truly are, while the prolonged wet appearance on nylon can give the opposite impression. Substrate-specific features, such as the central pooling pattern observed on silk or the complete absorption seen in cotton, may be mistaken for mechanisms of dynamic impact

rather than artefacts of textile behaviour. Temperature also played a clear role: brighter, more vivid stains in cold environments and darker, denser stains under heat have implications for evaluating clothing stored in vehicles, outdoor spaces, and temperature-controlled interiors.

By characterising the combined influence of textile properties, droplet volume, and environmental temperature under controlled conditions, this study provides practical reference information for forensic practitioners. These findings emphasise the importance of evaluating bloodstains in conjunction with fabric structure and environmental history to improve the accuracy and reliability of BPA interpretations involving garments and textile evidence in Forensics.

5.8 Limitations

This study was conducted under controlled laboratory conditions, which do not fully replicate the complexity of real casework environments. Only four fabrics were examined, representing a limited subset of textile types commonly encountered in forensic investigations. All experiments were performed without a second surface beneath the fabrics, meaning compression, garment layering, and real-world contact effects were not modelled. Although Keyence microscopy allowed qualitative depth assessment, the instrument limitations prevented precise quantification of penetration depth. Statistical comparisons were limited to descriptive patterns, as the study focused on experimental characterisation rather than hypothesis testing. The use of defibrinated horse blood, although consistent with BPA research practices, may not fully reproduce clotting behaviour and viscosity changes associated with human blood. These factors must be considered when extrapolating the findings to Forensic casework scenarios.

5.9 Future Work

Future studies should expand the textile range to include additional fibre types, blended fabrics, and varied weave densities to better reflect the diversity of garments encountered in forensic casework. Incorporating second-surface effects, layered fabrics, and garment compression would provide a more realistic representation of how stains develop during wear or impact. Controlled manipulation of blood age, clotting state, and dilution would allow deeper examination of time-since-deposition indicators. Higher-resolution instrumentation, including 3D optical profilers or confocal microscopy, could overcome current depth-measurement limitations and

allow quantitative analysis of stain penetration. Human blood, where ethically and legally permitted, would strengthen biological realism. Further research on the influence of droplet angle, velocity, and dynamic contact would also broaden the applicability of these findings. Such developments would build toward a comprehensive framework for interpreting bloodstains on textiles across a wider range of forensic contexts, ultimately strengthening the evidentiary value of BPA in forensic casework.

6. Conclusion

This study demonstrates how fabric structure and environmental temperature shape the drying behaviour and visual development of passive bloodstains on common textile substrates. By examining four fabrics under controlled thermal conditions, the work establishes that bloodstain formation on garments cannot be interpreted reliably without considering the properties of the underlying textile. The findings reinforce the principle that clothing introduces a level of variability not present on smooth reference surfaces that are often used in foundational BPA research.

The present study contributes to the wider forensic literature by offering clearly documented, substrate-specific observations that can support practitioners when evaluating stains on clothing recovered from casework. Understanding how textiles influence the appearance, size, colour and drying progression of a stain can improve the accuracy of assessments relating to timing, storage conditions, and potential movement of garments before or after an incident.

The study highlights the importance of considering textile structure and environmental history when assessing the age, appearance or formation of a stain. Continued research across broader textile types, fluid conditions and environmental contexts will further strengthen the evidential value of BPA applied to garments and other fabric-based materials. In this way, the work provides a foundation on which more comprehensive textile-focused BPA studies can be developed, improving both interpretive confidence and scene reconstruction reliability within forensic practice.

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