

Research Paper

Evaluation of the detectability of different ages of bloodstains on fabrics in different washing conditions and at various wavelengths

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ABSTRACT

Purpose: The detection of bloodstains at crime scenes is extremely useful in forensic investigations. This study aimed to investigate the effects of washing temperature, fabric type, fabric color, and stain age (time from staining to laundering) on the detection and identification of bloodstains on fabrics after washing.

Material and method: A total of 240 fabrics (4 different colors and 5 different types) were stained with blood and washed in 4 different washing temperatures with 3 different lag times. The evaluations of fabric images were performed using the FLS system (Forenscope-Mobile Multispectral UV-VIS-IR Imaging Systems®) on a total of 1200 images using 5 different wavelengths and filter options. The bloodstained areas of the fabric pieces were then excised, and the hemoglobin presence was analyzed using the SERATEC® HemDirect hemoglobin test.

Results: The analyses of laundered samples using the FLS system revealed that the best images were obtained from velvet, cotton fleece, denim, and polyester fabrics, in that order. Except for polyester fabrics, the SERATEC® HemDirect hemoglobin screening test, which was used to detect bloodstains on fabrics, showed positive results after washing at low temperatures (approximately 15 °C and 30 °C). At higher temperatures (60 °C and 90 °C), the SERATEC® HemDirect hemoglobin test yielded negative results.

Conclusion: The fabric type and color played a crucial role in stain detection using the FLS system on the laundered fabrics. The FLS system and the SERATEC® HemDirect hemoglobin test revealed that stain age had a limited effect on the stain's detectability.

1. Introduction

Detection of biological stains at the crime scene and DNA extraction from these samples are critical steps in identifying the offender or victim of the incident. Bloodstains are among the most common stains found at crime scenes and have a significant forensic value in terms of criminal justice.^{1–3} Bloodstains can often be found on different fabrics, such as clothes, sheets, or blankets, belonging to individuals involved in a forensic case. The characteristics of fabrics, including thickness, weaving pattern, content, and color, affect the formation of bloodstains.⁴ These factors may influence the visibility and detectability of bloodstains at the crime scene.

Even when blood is visible at the crime scene, the visibility of bloodstains may need enhancement if it is present in less quantity, not clearly visible on the surface, or has been dragged by objects, such as

shoes.⁵ In addition, bloodstained clothing or items may be destroyed or washed after a while to hide the crime.¹

Although there are numerous methods for detecting bloodstains, especially in cases where samples are scarce, important evidence such as DNA may be compromised by DNA degradation due to destruction of the bloodstained samples.^{5–7} The most commonly observed body fluid at a crime scene is blood.⁷ Many crimes, ranging from murder to assault, involve blood.⁸ Bloodstains can absorb light at wavelength 415 nm.⁹ The Forenscope-Mobile Multispectral UV-VIS-IR Imaging Systems (FLS) system readily allows visualization of the location of the stains and documentation as scientific evidence, which can be then presented to the court.¹⁰ The FLS system is a nondestructive and noninvasive method based on the absorptive and photoluminescent properties of the biological stains.^{10,11} As it permits rapid scanning of large surfaces at the crime scene, using it before advanced verification methods saves time.⁶

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After the stain has been located, various methods are employed to confirm the stain prior to performing DNA analysis. Immunoassay methods, which are frequently used in forensic laboratories, are performed for the quick and reliable detection of the presence of hemoglobin.^{7,12} The visible spectrum of hemoglobin consists of three main peaks. In fresh bloodstains, hemoglobin appears as oxyhemoglobin, with the strongest peak of oxyhemoglobin at 415 nm, known as the “Soret” band or “γ” band.¹³

The increase in societal awareness has led to the increase in the tendency of perpetrators to disrupt the crime scene. Criminals frequently wear gloves to avoid leaving their fingerprints or DNA, and they use bleach to clean up the crime scene. A crime scene can be cleaned to destroy evidence of the incident, and surfaces that serve as evidence can be painted.¹⁴ Many studies on the detectability of semen and bloodstains (Table 1) on washed clothing were conducted to gain further insights into the clarification of such cases.^{15–20} In two studies, Karadayı et al. reported that semen stains on fabrics can be detected even after washing,¹⁵ and the detection rate increases as the age of the stain increases.¹⁶ However, further studies are needed to determine the detectability of bloodstains in a wider range of fabric types and colors after washing in different lag times and washing conditions. Arjun et al. found that the detectability of bloodstains after washing was highest in cotton and brasso fabric types.¹ However, little is known about the detectability of a bloodstain after machine washing a textile product.¹⁸ Studies conducted on bloodstains have primarily used luminol to investigate the visibility factors in washed fabrics. To the best of our knowledge, no detailed study has been conducted to investigate the effect of the lag times of bloodstains on different types of fabrics prior to washing in determining the stain location and confirming the stain using the FLS system.

In this study, we aimed to reveal the effect of stain lag times before washing on the detection and identification of bloodstains on laundered clothes. Using a newly developed UV-VIS-IR imaging system with IR feature, we also aimed to comprehensively (four different colors of each of five different fabrics) confirm the role of other factors (washing temperature, fabric type, and color) that have been shown to have an effect on the detection of washed bloodstains on fabrics.

2. Materials and methods

2.1. Sampling

A sample of 25 cc of fresh blood was taken from 6 healthy adult volunteer donors. To mimic blood samples obtained from a crime scene, the samples were not placed in EDTA tubes, and bloodstains were quickly created with Pastor pipettes without allowing blood to coagulate. The detectability of the bloodstain was evaluated based on the lag times of bloodstains, washing temperatures, and fabric types and colors. Given the possibility of encountering fabrics with different structural properties at a crime scene, commonly used fabric types and colors with varying properties were preferred in the study. A bloodstain was created using 20 different fabrics, including 5 different fabric types (cotton, polyester, denim, velvet, and fleece) and 4 different color tones (2 light and 2 dark). Taking into account 3 different lag times and 4 different washing temperatures, 240 pieces of fabric ($3 \times 4 \times 20 = 240$) were collected. To determine the location of the bloodstains that would form on each fabric after washing, a circle with a diameter of 5 cm was drawn using a pencil, which would not be erased in water. The midpoint of the marked area on the fabrics was stained with 500 µL fresh blood. Considering the delays in incident reporting and the availability of biological evidence, the fabrics were dried at room temperature after staining and kept in 3 different paper envelopes for 24 h, 1 week, and 1 month, respectively, prior to performing the washing protocol. As a result, three groups were obtained. The graphs were created using IBM Statistical Package for the Social Sciences 25.00.

This study was approved by the Clinical Ethics Committee of the

Table 1

Forensic studies on washed bloodstained fabrics.

Studies	Deposition Time	Fabric Types	Washing Program	Analysis Methods
This Study	24 h	Cotton	~15 °C	FLS system
	1 week	Denim	30 °C	HemDirect test
	1 month	Polyester	60 °C	
		Polar	90 °C	
Arjun and Ashish ¹	24 h	Velvet	Waiting-hand-washed	Kastle–Meyer
		Cotton		
		Cotton		
		brasso		
		Rubiya		
		cotton		
		Polyester		
		Linen		
		Linen		
		cotton		
Gupta et al. ²	2–3 days	mix	Hand-washing	Tetra-methyl Benzidine test
		Terrycott		
Elder et al. ¹⁸	1 day	Cotton	20 °C	Luminol Test Luminol DNA recovery STR typing
		denim		
	1 week	Polyester	30 °C	
		Cotton	60 °C and	
		jarsey	95 °C	
		Micro fleece		
Mustaq et al. ¹⁰	24 h	Cotton	Hand-washing at room temperature	Hemastix Kastle–Meyer Leucomalachite green Leucomalachite green
		Polyester		
		Cotton-polyester		
		Keti		
		Tarawere		
		Tarawere		
		Lawn		
		Bosky		
		Khaddar		
		Wool		
Hofman et al. ¹⁸	24 h	Silk	30 °C and 60 °C	Combur Luminol DNA recovery STR typing
	48 h	polyester		
	72 h	Cotton		
	4,5 day	Polyester		
	7 day			
Kitpipit et al. ¹⁹	24 h	Cotton	20 °C	Kastle–Meyer STR typing
		Polyester	40 °C	
		Denim	60 °C	
			95 °C	
Sapan et al. ²⁰	60 min	Nylon	40 °C	Luminol DNA recovery
		Cotton	60 °C	
			95 °C	
Stajanović ²³	6 h	Cotton	30 °C	Hemastix Bluestar
	24 h		60 °C and 95 °C	
	72 h			
	10 days			
	30 days			

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2.2. Laundering protocol

The bloodstained fabrics, which were kept for 24 h, 1 week, and 1 month under appropriate conditions, were washed in a household washing machine (Bosch Maxx 8) at 1000 rpm and 4 different temperatures: ~15 °C (tap water temperature at the time of laundering), 30 °C, 60 °C, and 90 °C. Each bloodstained fabric was washed with 65-mL enzyme-based liquid laundry agent (Bingo liquid laundry detergent)

by grouping the fabrics with the same lag time and color tone. No detergent was used at 15 °C. To prevent cross contamination among the groups, the washing machine was run without fabrics between washes. Fabric softener and laundry bleach were not used in any of the washes.

After the washing process, the fabrics were dried at room temperature overnight and the marked areas were examined.

2.3. Testing for blood stains

To determine the persistence of the bloodstains after washing, each stain (240 samples) created within the marked area on each fabric was investigated using the FLS system. The bloodstained area was excised, and the presence of hemoglobin was determined using the SERATEC® HemDirect hemoglobin test (SERATEC Gesellschaft für Biotechnologie mbH, Göttingen, Germany).

2.4. Examination of bloodstains with the FLS system (Forenscope)

An advanced forensic light source system was used to determine the location of bloodstains on fabrics and investigate stain persistence after the laundering procedure. The Forenscope-Mobile Multispectral Tablet® (Forenscope EUROPE) was used for the examination, which has a built-in imaging, recording system, and portable forensic light source system with Android operating system that can emit light at different wavelengths, allowing the use of different filters. For each sample, the following five different lights + filter options were used:

- White Light (6500 k) + VIS filter + Color Camera Mode
- Violet Light (410–420 nm) + BP415 Filter (BandPass 415 nm Filter) + Color Camera Mode
- Violet Light (410–420 nm) + BP415 Filter (BandPass 415 nm Filter) + Monochrome Camera

Mode.

- Violet Light (410–420 nm) + BP415 Filter (BandPass 415 nm Filter) + Invert Camera Mode
- Infrared 840–860 nm + IR filter + Monochrome Camera Mode

All fabrics were photographed and recorded using Mobil Multi-spectral UV-VIS-IR Imaging Systems®. A total of 1200 ($5 \times 240 = 1200$) images were obtained for 5 different shots based on the different combinations of filters and wavelengths of light for each sample. To take photographs from a standard distance and prevent external light from affecting the images, a dark room supplied with the system and customized for the device was used. After photographing, the images from the camera were transferred to a computer in JPEG format and sorted according to variable characteristics to facilitate evaluation. From the obtained images, three researchers (BK, SK, and DO) selected four reference images (negative, weak, moderate, and strong images). Based on the reference images, the same three researchers independently evaluated and categorized the captured images into the four groups, taking into account the visibility of the stain in the images. If a unanimous decision was not made for a sample, the opinion of the majority of the researchers for that image was considered. For example, if the first researcher evaluated the image as strong, the second researcher as moderate, and the third researcher as strong, the result was recorded as strong. To assess the intraobserver agreement, 60 randomly selected images were reevaluated by the first author (DO). The same images were evaluated separately by the second and third authors (SK and BK), and interobserver agreement was tested on 120 assessments. Cohen's kappa test²¹ was performed to calculate the intraobserver and interobserver reliability.

2.5. Confirmatory blood test and evaluation

The presence of hemoglobin on the washed bloodstains in all groups was analyzed using the SERATEC® Hemdirect test. According to the kit manufacturer's (SERATEC) instructions, the cut underwear pieces were kept in 1 ml buffer solution for 10 min. Approximately 100 µl (three drops) of this solution was added to the sample well. Results were noted after the end of the 10-min incubation period. The presence of a pink line in the test region (T) indicates that the test is positive. Results based on the status of the pink line was interpreted according to modification of Holtkötter et al. Classification.²² These categories were defined as “++++,” “+++,” “++,” and “+” in the presence of the strong T-line with sharp edges, T-line with sharp edges, light T-line with one sharp edge, and faint T-line, respectively, and clear result window was noted as negative.

3. Results

Cohen's kappa value used for measuring intraobserver agreement for evaluation stages was 0.93, indicating almost perfect agreement. Likewise, interobserver agreement (kappa value: 0.74) was substantial accordance.

The results of the FLS system examinations on the samples are shown in the Supplementary, and the results of the SERATEC® Hemdirect hemoglobin test are shown in Table 2.

3.1. Forensic light source system

The results obtained from the FLS system analysis of the bloodstained fabrics washed with different washing protocols revealed that the strongest to the weakest images were obtained in velvet, cotton, polar fleece, denim, and polyester for all lag times (Fig. 1A).

In terms of the prominence of bloodstains after washing, the FLS system produced a better image in light-colored fabrics compared to dark-toned fabrics. When all storage times and washing temperatures were considered, cotton white and cream, velvet white and cream, and fleece white and cream colored fabrics produced the best results (Figs. 1–3).

It was observed that the rate of detectability and image quality using

Table 2

HemDirect test evaluation results for washed bloodstained fabrics categorized according to fabric type, washing temperature, and stain age.

Fabric Type	°C	Blood Stain Storage Time		
		24 Hours	1 Week	1 Month
Cotton	~15 °C	++++ ^a	++++	++
	30 °C	–	+	++++
	60 °C	–	–	–
	90 °C	–	–	–
Polyester	~15 °C	+++	+++	++++
	30 °C	+	–	–
	60 °C	–	–	–
	90 °C	–	–	–
Denim	~15 °C	++++	+++	+++
	30 °C	+	+++	++++
	60 °C	–	–	–
	90 °C	–	–	–
Velvet	~15 °C	++++	+	++
	30 °C	+	–	+
	60 °C	–	–	+
	90 °C	++++	–	–
Polar	~15 °C	+++	+++	++
	30 °C	–	+	++++
	60 °C	–	+	–
	90 °C	–	–	–

^a “++++,” “+++,” “++,” “+” and “–” indicate strong positive, positive, weak positive, very weak positive and negative results for HemDirect immun-assay, respectively.

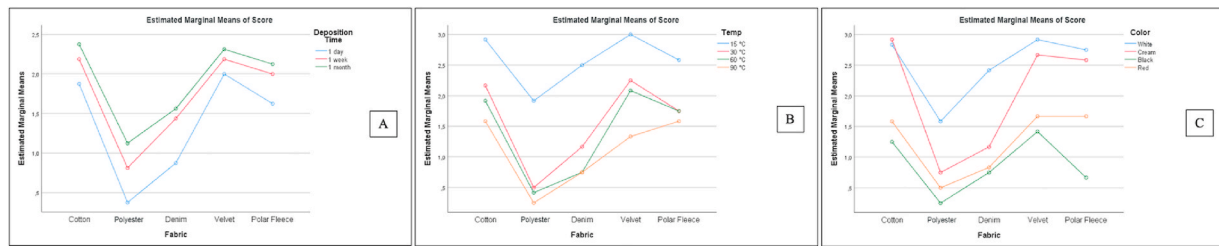


Fig. 1. The effects of fabric color (A), washing temperature (B), and lag periods of the stain (C) on the estimated marginal means in different fabric types using the forensic light source system (ForenScope). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Storage Time	Fabric Type and Washing Conditions							
	B-P-W-15°C (Polyester fabric, White color, washed at about 15°C)	B-D-G-15°C (Denim fabric, Gray color, washed at about 15°C)	B-PF-CR-30°C (Polar Fleece fabric, Cream color, washed at 30°C)	B-D-SB-30°C (Denim fabric, Soft Blue color, washed at 30°C)	B-PF-W-60°C (Polar Fleece fabric, White color, washed at 60°C)	B-C-R-60°C (Cotton fabric, Red color, washed at 60°C)	B-PF-CR-90°C (Polar Fleece fabric, Cream color, washed at 90°C)	B-P-W-90°C (Polyester fabric, White color, washed at about 90°C)
24 HOURS								
1 WEEK								
1 MONTH								

Fig. 2. Examples of the forensic light source (ForenScope) showing bloodstains on fabrics based on differences in laundering procedures, lag periods, fabric types, and shooting mode.

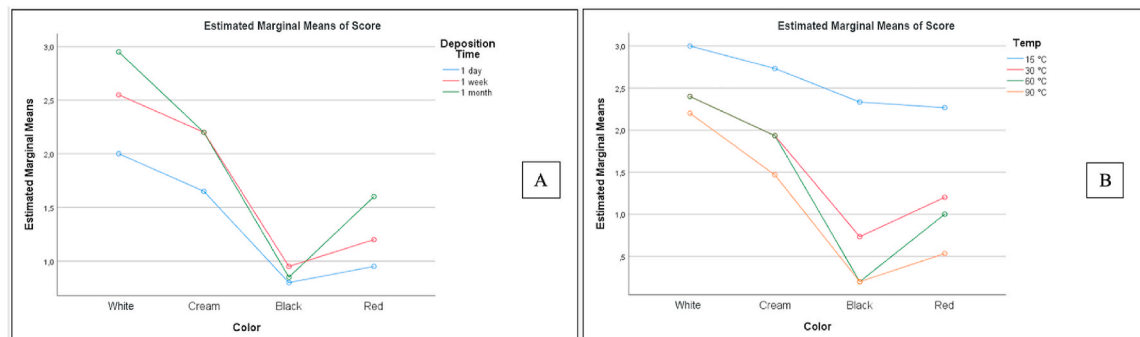


Fig. 3. The effects of lag periods (a) and washing temperature (b) on the estimated marginal means in different colors of fabrics using the forensic light source system (ForenScope) examinations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the FLS system decreased in all fabric types and tones with the increase in the washing temperature (Supplementary, Fig. 1B).

The location of the bloodstain could be determined after the laundering procedure in 72% images evaluated in the study (Supplementary). Considering the washing conditions and lag times of bloodstains, the least successful FLS system analysis results were observed in black-colored fabrics (Fig. 1C; Fig. 3A and B). Images were usually obtained in dark-colored fabrics using IR light (Fig. 4).

The lag time of the bloodstained fabrics before washing had an effect on the quality of detectability in the FLS system analysis. The best results

obtained by the FLS system analysis were observed in laundered bloodstained fabrics with 1 month of lag time (Fig. 1A; Fig. 2; Fig. 3A; Fig. 5).

When the images displayed and recorded for each sample using different wavelengths and filter systems of the Forenscope device were evaluated, the best images were obtained using the following parameters:

- Violet Light (410–420 nm) + BP415 Filter (BandPass 415 nm Filter) + Color Camera Mode,

Conditions	Fabric Type				
	Cotton	Denim	Velvet	Polar	Polyester
R-30-1M (Red fabric washed at 30 °C degrees after standing for 1 month)					
R-60-1M (Red fabric washed at 60 °C degrees after standing for 1 month)					
R-90-1M (Red fabric washed at 90 °C degrees after standing for 1 month)					

Fig. 4. Forensic light source (ForenScope) examinations at IR wavelength of bloodstains on different dark-toned fabric types after various laundering procedures.

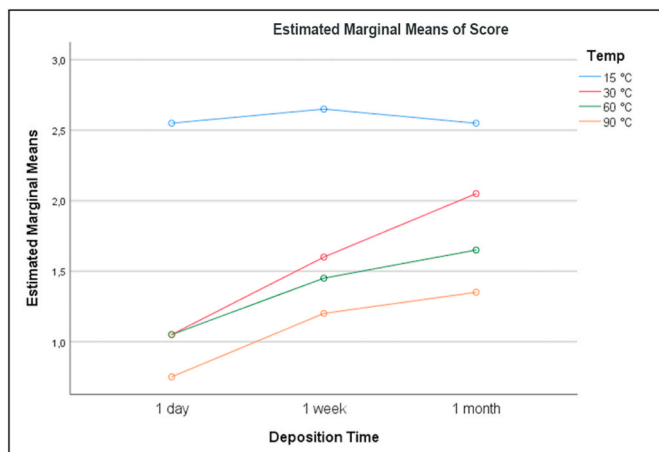


Fig. 5. The effect of washing temperature on the estimated marginal means in different lag periods investigated using the forensic light source system.

- Violet Light (410–420 nm) + BP415 Filter (BandPass 415 nm Filter) + Monochrome Camera Mode
- Violet Light (410–420 nm) + BP415 Filter (BandPass 415 nm Filter) + Invert Camera Mode options (Sup.).

3.2. Serological testing

Regardless of the stain retention time, the SERATEC® HemDirect hemoglobin test produced a strong positive result in all fabric types when no detergent was used at water temperature of ~15 °C.

With a 1-month lag period, strong positive results were observed in cotton, denim, and fleece fabrics that were washed with detergent at a water temperature of 30 °C, whereas weak positive results were observed in velvet fabric (Table 2).

It was observed that when the water temperature was higher than 60 °C, the bloodstain produced a negative result regardless of its age (Table 2).

4. Discussion

Blood and blood-contaminated items are frequently encountered during a crime scene investigation, and extracting DNA from such samples in the later stages of the investigation is critical to procure evidence regarding the incident and the criminal. However, criminals often try to clean up the blood at the crime scene in order to destroy evidence, and in some cases, they attempt to destroy evidence by washing bloodstained clothing. Previous studies based on the determination of location of the bloodstain after the washing process are usually conducted using chemical substances, such as luminol. Although the FLS systems have been shown to be effective in detecting biological stains,^{11,12} the number of studies that employed the FLS system is limited. Furthermore, the effect of blood persistence and stain lag time of washed clothes on the detection rate of evidence has not been fully explored. Therefore, we used a cutting-edge Forenscope light source system (Forenscope), which is more efficient than the traditional forensic light source systems.

In the present study, the best FLS system image was obtained in velvet, cotton, polar fleece, denim, and polyester fabrics. Gupta et al. obtained pronounced result in hand-washed cotton fabric using the luminol test.² In the study conducted by Edler et al. using luminol, after washing some fabric by hand and some by machine, the least blood residue was observed in micro fleece fabrics (24.2%), whereas macroscopic bloodstains were detected in all the cotton denim samples.¹⁷ Among all samples, the presence of blood was detected macroscopically in 57.2% samples after the washing process, whereas a reaction rate of 95.9% was observed in all samples by the luminol technique; this rate was 88.6% in the control samples.¹⁷ In the present study, the stain location was detected after washing in 72% evaluated images. The image quality obtained after washing may have been influenced by the

amount of blood used in the formation of the stains. Edler et al. used 10 mL of blood, whereas 500 mL of blood was used in the present study. Stojanović et al. reported positive results with Hemastix® and Bluestar® in washings at 30 °C, 60 °C, and 90 °C in cotton fabrics.²³

Edler et al., citing Grifford et al., reported that after 9 h of soaking in still water and 30 min of washing in moving water, no macroscopic bloodstain was observed on the fabrics with blood that were kept from a few seconds to a maximum of 9 h. Edler et al. reported that lag times of 1 day and 1 week do not affect the reaction to luminol after washing.¹⁷ In our study, stain images were obtained in 62%, 72%, and 81% fabrics that were washed after 24 h, 1 week, and 1 month of lag period. Based on these results, it was concluded that the percentage of detectability increases with the lag time prior to washing. Hofmann et al. reported better stain images because the lag time before washing increases in their study using the luminol method. A weaker luminol reaction was reported after washing in fabrics that were kept for 24 h than in fabrics that were kept for 3 or more days.¹⁸

In the present study, when all the lag times and washing temperatures were considered, favorable results could not be obtained in the polyester fabric owing to its poor absorbency and moisture retention properties. Similarly, in the study by Edler et al., the least chemiluminescence intensity was observed in polyester fabrics, probably due to the failure of hemoglobin adhesion to the polyester.¹⁷ Our study results are consistent with this finding.

A study that used poly light found that the stain image quality was poor due to high absorption of blood by polar fleece; however, a better image was obtained on light-colored cotton fabric.²⁴ Hofmann et al. used the luminol method and reported that stronger fluorescence was obtained in light-colored fabrics.¹⁸ The researchers suggested that fluorescence was absorbed in black-colored fabrics and other methods should be used in such cases.¹⁸ Similarly, in terms of the persistence of the bloodstain, a better image was obtained in this study with the FLS system in light-tone fabrics than in dark-tone fabrics after washing. Another study found that using infrared light sources was beneficial in dark fabrics.²⁵ According to Chun-Yen Lin et al. study, which used 10 different types of black fabrics, stains were visible up to 1/8 dilution with IR.²⁵ However, Sterzik et al. reported that IR enhances the visibility of undiluted blood, but stain images cannot be obtained in diluted blood.²⁶ In our study, the decreased detectability of stain age with IR, especially in dark-colored fabrics washed at high temperatures, supports this finding. Moreover, no favorable stain images could be obtained on the denim fabric at any of the three temperatures.

Many factors influence the effectiveness of the FLS systems, including washing temperature, drying time, type of washing machine, type of sample investigated, fabric color and type, quantity of bloodstain, and time elapsed between the crime commission and crime scene investigation.^{10,24} Edler et al. could not confirm the effect of washing water temperature on macroscopic cleaning. Although the lowest chemiluminescence was observed with washing at 95 °C, it was reported that stains remained more stable in samples washed at 60 °C.¹⁷ In the study conducted by Hofman et al. using luminol, it was reported that there was no major difference between washing of textile products at 30 °C and 60 °C, and they also reported that detectability at 95 °C was significantly reduced.¹⁸ In their study on cotton fabric, wood, and tile surfaces, Miranda et al. reported that fluorescence intensity varies with sample age. The fluorescence was lower when the samples were moist, and the fluorescence was stable for 60 days on dry samples regardless of the surface where the stain sample was located.²⁷ In the present study, stain visibility did not differ significantly between fabrics washed at 30 °C and 60 °C for the 24-h lag period, but some difference was observed for 1-week lag times. Considering the lag period of a maximum of 1 week, our findings of 1-week lag times were consistent with those of Hofman et al.¹⁸ In the present study, the difference in fabrics subjected to a 1-month lag period was considerably higher than the others and was significantly reduced at 95 °C. We found that increase in washing temperature decreased the rate of detectability and image quality using the

FLS system.

Bloodstains are the most visible at 415 nm with yellow glasses and at 410 nm without glasses.²⁴ Although we obtained a favorable image of blood at a wavelength of 415 nm, this was not the sole wavelength for effective forensic blood imaging. Favorable results were obtained using the violet and the monochrome option of the FLS System. Bloodstains on dark-colored fabrics should be located with an IR light.

In some studies, a greenish discoloration has been reported after washing. Similar observation has been made in dried samples for up to 3 days by Edler et al. and in bloodstained fabrics kept for 1 month in our study. Adair and Shaw in their studies using luminol suggested that this coloration could be due to the moisture in samples before washing.²⁸ Another hypothesis is that the laundry detergents can cause degradation of blood proteins, and the released sulfur compounds make the stain appear green,¹⁷ which seems more likely given that the stains are thoroughly dried before washing.

Enzyme-based detergents can remove proteins, such as hemoglobin. In a study conducted by Arjun et al.¹ with five different enzyme-based detergent brands, it was determined that Ariel® was the best detergent for removing stains in the washing of bloody fabrics. As the washing agent used in our study (Bingo) is also an enzyme-based detergent, this could explain the reason behind the negative result of the SERATEC® HemDirect hemoglobin test, especially at the high temperatures.

As shown in Table 2, positive results were obtained by the SERATEC® HemDirect hemoglobin test after 24 h, 1 week, and 1 month of lag periods in all fabric types washed with detergent-free water at ~15 °C. Similar results were obtained in the FLS system analyses. This finding shows that a detergent-free wash at low temperature is substantially insufficient to completely remove bloodstains from any type of fabric.

The strong positive results obtained in cotton, denim, and fleece fabrics washed with detergent at 30 °C water temperature after a 1-month lag period suggested that washing such fabrics at this temperature does not completely remove stains after long-term lag periods. However, it has been determined that when the water temperature rises above 60 °C, the bloodstain yields a negative result regardless of the stain age. This finding demonstrates that washing bloodstained fabrics at high temperatures and with detergent can completely remove the stain. In the samples where the stain was visible after washing but negative result was with the SERATEC® HemDirect hemoglobin test, we suggest that this may be due to denatured hemoglobin, especially at high temperatures.

In addition to being nondestructive and less time-limited than other methods, the expansion of the spectrum with evolving technology makes the FLS systems more useful.

5. Conclusion

Based on the results of the FLS system analysis and the SERATEC® HemDirect hemoglobin test, it can be concluded that stain age has a limited effect on stain detectability. According to our findings, the fabric type and color were the most crucial features in the detectability of the stain using the FLS system on the laundered fabrics. Furthermore, the highest detectability was obtained at low washing temperatures and longer lag times, whereas bloodstains on light-colored fabrics were more detectable than on dark-colored fabrics. However, bloodstains that could not be detected at other wavelengths on dark fabrics could be detected by IR. The SERATEC® HemDirect hemoglobin test analyses revealed that the washing temperature was more significant factor than the lag time and fabric type.

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Declaration of competing interest

The authors declare that they have no known conflicting financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jflm.2023.102486>.

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