A Comparative Analysis of Commercially Available Protein and Peroxidase Reagents for Blood Detection and Enhancement on Laundered Clothing

Gabrielle Hartley & Dr. Claire Glynn, Ph.D.

Department of Forensic Science, Henry C Lee College of Criminal Justice and Forensic Science, University of New Haven, West Haven, Connecticut

Abstract

Blood on a suspect or victim's clothing is not uncommon in criminal cases involving violent incidents, and often these stains will be washed away in hopes destroy the evidence. This study aims to produce a comprehensive analysis of three protein based reagents and three peroxidase based reagents commonly used and commercially available for the detection of trace amounts of blood on laundered clothing. Enhancement reagents Hungarian Red, Coomassie Blue, Amido Black, luminol, Bluestar® Forensic Magnum, and aqueous Leuco Crystal Violet (LCV) were used to detect 100 μl human blood stains on varying fabric types and colors (white cotton, black cotton, blue denim, white polyester, and black polyester) at a range of blood dilutions (neat, 1:10, 1:100, 1:1000, 1:10000, 1:100000, 1:1 million) after laundering to determine the usability and sensitivity of the reagents. This study revealed that the peroxidase based reagents produced the greatest sensitivity on the natural fabrics, reacting positively down to a blood dilution of 1:1000. The protein reagents produced greater sensitivity on the synthetic fabrics, reacting positively down to a blood dilution of 1:10. Peroxidase stains relying on chemiluminescent properties rather than colorimetric results produced better results on the dark colored fabrics. The results of this study suggest the importance of laundered clothing as evidence and provides an analysis of these six reagents for blood detection on fabrics after blood evidence has been washed.

Introduction

In criminal and forensic investigations, the connection between a perpetrator and a victim can often be the most condemnatory piece of evidence an investigator can find. Blood evidence is common in cases of violent crime, and it is not unlikely that a suspect will try to destroy this evidence. Washing blood off of clothing results in dilute stains that are often difficult to detect.[1] The two main types of reagents that can be used for detection of trace amounts of blood are *protein* stains and *peroxidase* stains. Protein stains are those that react with amines or other groups found within blood proteins to produce colorimetric results. Many of these reagents are inexpensive, easy to use, and can generally be used on porous or nonporous substrates, including fabrics.[2] Examples of these stains include Hungarian Red, Coomassie Blue, and Amido Black. Peroxidase reagents are a group of reagents that react with the iron found in heme, a structural element of hemoglobin. The heme catalyzes the reaction between the dye used and an oxidizer, producing a quick color change in the presence of blood.[3] While some peroxidase reagents produce a colorimetric result, some form a chemiluminescent effect. Examples of peroxidase reagents for blood detection include aqueous Leuco Crystal Violet, luminol, and Bluestar® Forensic Magnum. Because other materials can produce these results (including some plants, cleaning materials, and metals), these methods are considered presumptive tests for the presence of blood.[3] They can provide a strong starting point for a later method to be used to confirm the presence of human blood.

Objectives

The objectives of this research was to:

1. Provide a comprehensive analysis of the optimal reagent to detect bloodstains on fabrics of varying colors and compositions
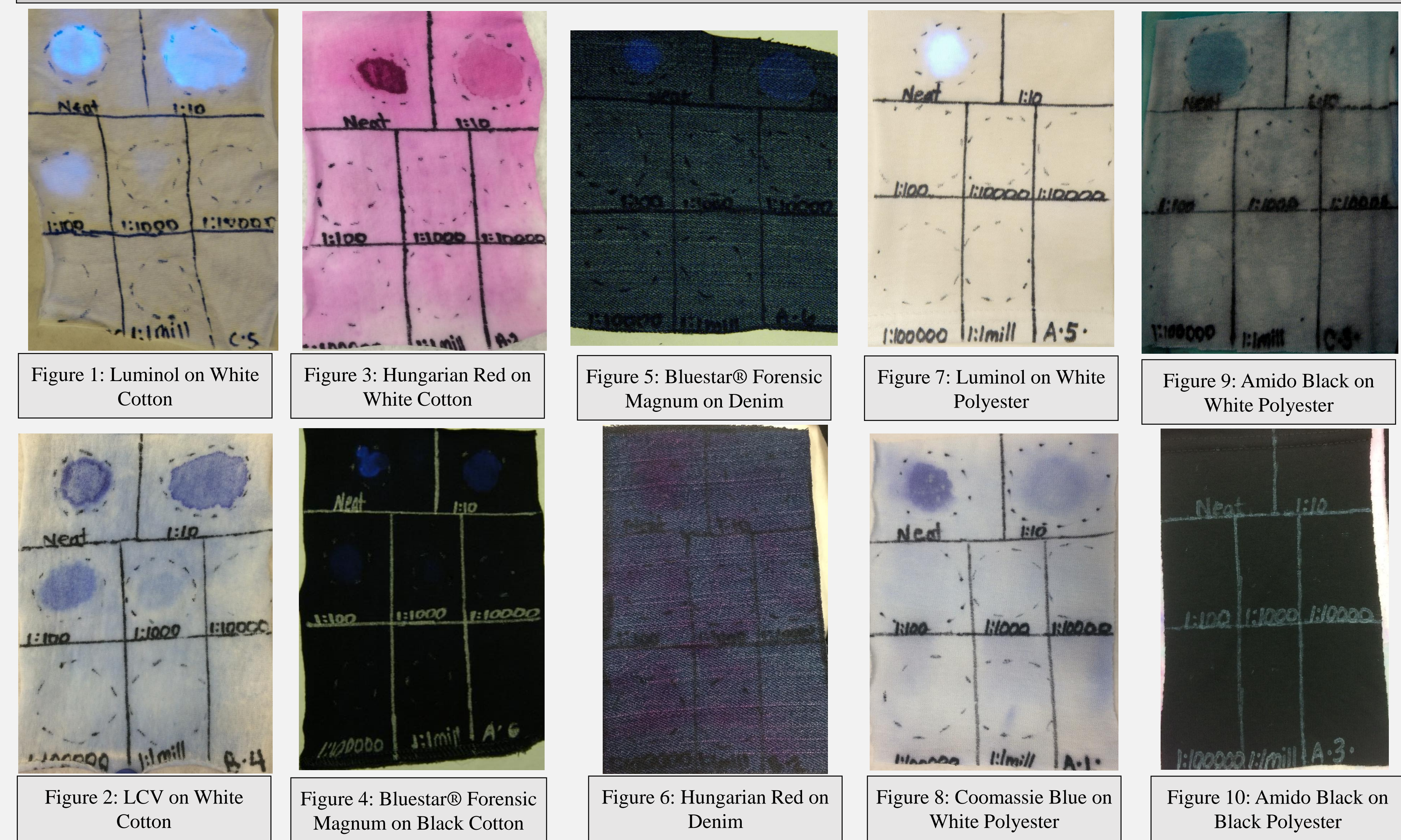2. Determine the sensitivities of each reagent on laundered bloodstains

Materials and Methods

Following informed consent and approval from the Institutional Review Board at the University of New Haven, venous blood was obtained from volunteers into sterile vacutainer EDTA vials and stored at 4 ℃.

Five fabric types were selected: white cotton, black cotton, white polyester, black polyester, and blue denim.

Six reagents were selected and purchased from Sirchie;
- Protein based; Hungarian Red, Coomassie Blue, and Amido Black
- Peroxidase based; Luminol, Leuco Crustal Violet (LCV), and Bluestar® Forensic Magnum

100μl of human blood was deposited onto each fabric type in a range of seven dilutions from neat to 1 in 1 million. Each sample was performed in triplicate and photographed prior to laundering. Following laundering with a standard detergent and washing cycle, each sample was enhanced and photographed following the manufacturer's instructions provided with each of the six reagents. The results of each reagent, dilution, and fabric type were compared using a scale from 0-4 (0 = no reaction; 4 = strong positive reaction).

Results

Figure 1: Luminol on White Cotton

Figure 3: Hungarian Red on White Cotton

Figure 5: Bluestar® Forensic Magnum on Denim

Figure 7: Luminol on White Polyester

Figure 9: Amido Black on White Polyester

Figure 2: LCV on White Cotton

Figure 4: Bluestar® Forensic Magnum on Black Cotton

Figure 6: Hungarian Red on Denim

Figure 8: Coomassie Blue on White Polyester

Figure 10: Amido Black on Black Polyester

	Neat						1:10						1:100						1:1,000					
	L	BFM	LCV	HR	CB	AB	L	BFM	LCV	HR	CB	AB	L	BFM	LCV	HR	CB	AB	L	BFM	LCV	HR	CB	AB
White Cotton	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✗	✗	✗
Black Cotton	✓	✓	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗
Denim	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✓	✓	✓	✗	✗	✗
White Polyester	✓	✓	✓	✗	✗	✗	✗	✗	✗	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Black Polyester	✓	✓	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗

Figure 11: This figure shows the sensitivity of each reagent on the various fabric types. A ✗ indicates no reaction, while a ✓ represents a positive reaction for each trial.
Key: L = Luminol ; BFM = Bluestar® Forensic Magnum ; LCV = Aqueous Leuco Crystal Violet ; HR = Hungarian Red ; CB = Coomassie Blue ; AB = Amido Black

Fabric	Highest Sensitivity
White Cotton	Luminol; Bluestar® Forensic Magnum; Aqueous Leuco Crystal Violet
Black Cotton	Bluestar® Forensic Magnum
Denim	Luminol; Bluestar® Forensic Magnum
White Polyester	Coomassie Blue; Amido Black
Black Polyester	Bluestar® Forensic Magnum

Figure 12: This figure shows the most sensitive reagent for each type of fabric. In this study, the most sensitive reagent is that which provided a result (1-4) at the lowest dilution.

Discussion

The results of the post-laundering enhancement of the neat blood and dilutions on the varying fabric types revealed the peroxidase based reagents (luminol, LCV and Bluestar® Forensic Magnum) to have the greatest sensitivities on the natural fabric types (white cotton, black cotton and denim) as they all reacted positively on these fabrics down to 1:1,000. However, when the protein reagents were tested, they revealed the greatest sensitivities (1:10) on the white polyester when compared to the peroxidase reagents, which only produced positive reactions on the laundered neat blood. As the protein based reagents are color reactions and are not based on chemiluminescence, their use on dark fabrics revealed indeterminate results.

Conclusion

The results of this study provide a valuable comparative analysis of commercially available blood enhancement reagents for use in the forensic investigations. The results suggest peroxidase based reagents to be the superior method for use on natural fabrics and chemiluminescent peroxidase reagents to be superior on all dark fabrics. Protein based reagents were best suited for use on synthetic fabrics. This information will serve as a valuable resource for forensic professionals in the future.

References

(1) Mushtaq, S., Rasool, N., & Firiyal, S. (2016). Detection of dry bloodstains on different fabrics after washing with commercially available detergents. *Australian Journal of Forensic Sciences*, 87-94.
(2) Farrugia, K. J., Savage, K. A., Bandey, H., & Niamh, D. (2011). Chemical Enhancement of Footwear Impressions in Blood on Fabric- Part 1: Protein Stains. *Science and Justice*, 99-109.
(3) Marie, C. (2008). Presumptive Testing and Enhancement of Blood. In T. Bevel, & R. M. Gardner, Bloodstain Pattern Analysis (pp. 275-296). CRC Press.

Acknowledgements