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

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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 to destroy the evidence. This study aims to produce a comparative 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, and Amido Black, 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, 1:10,000, 1:100,000, 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.

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 vacuumer EDTA vials and stored at 4°C. Five fabric types were selected: white cotton, black cotton, white polyester, black polyester, and blue denim. Six reagents were selected and purchased:

- **Protein based:** Hungarian Red, Coomassie Blue, and Amido Black
- **Peroxidase based:** Luminol, Leuco Crystal Violet (LCV), and Bluestar® Forensic Magnum.

100 µl 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).

Introduction

In criminal and forensic investigations, the connection between a perpetrator and a victim can often be the most commendatory 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. 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. 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 hemin, a structural element of hemoglobin. The hemin catalyzes the reaction between the dye used and an oxidizer, producing a quick color change in the presence of blood. 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. 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

Results

In Figure 1 the reagents are shown reacting on various fabrics. Figure 2 shows the dilution of the reagents. Figure 3 shows the results of using Luminol on White Cotton, Hungarian Red on White Cotton, and Bluestar® Forensic Magnum on Black Cotton. Figure 4 shows the results of using Luminol on White Cotton, Hungarian Red on White Cotton, and Bluestar® Forensic Magnum on Black Cotton. Figure 5 shows the results of using Luminol on White Cotton, Hungarian Red on White Cotton, and Bluestar® Forensic Magnum on Black Cotton. Figure 6 shows the results of using Luminol on White Cotton, Hungarian Red on White Cotton, and Bluestar® Forensic Magnum on Black Cotton.

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


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