Technical Note

Determining the Sensitivity and Reliability of Hemascein

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Abstract: Some of the most common tests for the detection of latent bloodstains include luminol, Bluestar, and fluorescein. Hemascein is a relatively new fluorescein-based method that uses the chemiluminescent reaction between fluorescein and the heme to detect latent blood. At present, few studies have assessed the sensitivity and reliability of Hemascein. The current experiment attempted to address this issue. Human blood concentrations (neat, 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, 1:1,000,000) were deposited on a variety of surfaces (linoleum, wood paneling, whiteboard, porcelain tile, and carpet) and then tested with Hemascein. We observed Hemascein to react with the greatest reliability on blood dilution ranges of 1:1,000 to 1:100,000. Hemascein was found to be most sensitive and reliable on light-colored, smooth, flat surfaces. It was also reliable and sensitive to neat (1:1) and 1:10 dilutions of blood on dark carpet. A benefit of Hemascein is the relatively few chemical safety issues associated with its use. A drawback is a high degree of background staining if sprayed improperly. Experimental work to assess the effect of Hemascein on subsequent DNA analysis is recommended.

Introduction

Bloodstain pattern analysis has been an important investigative tool since the mid-1800s. As such, tests and methods that detect bloodstains have evolved into the tests and methods in

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use today. These include methods of detecting latent bloodstains (i.e., bloodstains that are not visible to the naked eye).

A bloodstain is formed when blood is deposited on a surface [1]. The analysis of bloodstain patterns can result in crime scene reconstruction relevant to the spatial origin of the blood, location of the bloodletting event, and movement of the persons involved in the bloodletting event [2]. Yet, it is common for bloodstains to decompose over time, be cleaned up, or otherwise be invisible to the unaided eye. The ability to discover latent bloodstains such as these is often important to crime scene investigation and reconstruction. From this need came the development of bloodstain discovery techniques such as luminol, Bluestar Forensic, and fluorescein [3, 4].

Luminol is a widely used bloodstain discovery chemical. It uses a chemiluminescent reaction catalyzed by iron in the blood [5]. It has a recorded sensitivity of at least 1:100,000 [2], is commercially available, and easily prepared at a crime scene. It has also been shown that luminol does not necessarily compromise the integrity of bloodstain patterns or genetic information [2, 6]. However, luminol requires almost total darkness and luminesces for a short time only, making photography difficult. It also has a short shelf life and must be prepared and used at the scene. Further, multiple applications of luminol (as it is prepared as a liquid and sprayed onto a surface) to a bloodstain can potentially dilute a bloodstain and alter its shape. Luminol has also been observed to luminesce in the presence of fresh bleach, strong metal ions, and strong peroxidases, such as horseradish [5]. Luminol also has associated health considerations. It has been known to cause chemical burns, which introduces increased costs associated with scene clean up or compensation for damaged products [7].

Bluestar Forensic, like luminol, is a liquid that is sprayed. Also like luminol, Bluestar uses peroxidase-like activity of blood as a catalyst to produce chemiluminescence. Its luminescence is apparently brighter and longer than that of luminol [8] and it has a shelf life of several days. It has also shown to be more reliable at detecting latent blood that has been cleaned with bleach [5]. As a luminol-based product, Bluestar has been associated with the aforementioned health hazards.

A newer discovery technique is Hemascein, which is a fluorescein-based chemical. Fluorescein is a chemical compound that is reduced to fluorescin when combined with water. Fluorescin is then sprayed on a surface, followed by an

application of hydrogen peroxide (H_2O_2). The chemiluminescent reaction between H_2O_2 and fluorescin is catalyzed by heme. Hemascein has been used at a crime scene to detect latent fingerprints developed in blood on a vertical surface where, 18 months later, these prints still reacted to Hemascein, were photographable, and had comparable ridge detail [6]. Although Hemascein can be a valuable tool for bloodstain detection, the reliability and sensitivity of Hemascein are not thoroughly understood. The following experiment will address this gap in knowledge by testing the null hypothesis that Hemascein will not react with blood on surfaces commonly found at crime scenes. To test this hypothesis, we used Hemascein to discover fresh human blood deposited on contrasting surfaces (linoleum, wood paneling, whiteboard, porcelain tile, carpet) in a range of concentrations (neat, 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, 1:1,000,000).

Materials and Methods

Blood

A phlebotomist drew blood intravenously into sterile tubes, without preservatives or anticoagulants. This blood was used fresh, prior to clotting.

Surfaces

Experimental surfaces included (1) linoleum (Decorative Panels International, Toledo, OH, white colored, product # 346-428, 60.96 cm x 60.96 cm); (2) wood paneling (Traffic Master, Norwalk, CT, redwood colored, product # 361-057, run #25.01.2010, 30.48 cm x 30.48 cm); (3) whiteboard (Decorative Panels International, Toledo, OH, Bluelinx Paneling, product # 709-106); (4) porcelain tile (US Ceramic Tile Co., Miami, FL, black colored, product # U759-44-1M, 10.795 cm x 10.795 cm); and (5) Olefin carpet (Shaw, Dalton, GA, charcoal colored, product # 17357, 45.72 cm x 68.58 cm).

Experimental Design

Before the experiment, each surface, except carpet, was cleaned with distilled water and dried with a paper towel. Blood was then drawn and transferred to a sterile 50 mL beaker. Blood was taken from the beaker with a glass Pasteur pipette, and one drop (30 microliters ± 1 microliter) was deposited onto each surface in the area designated for neat blood (Figure 1). Neat blood was diluted with sterile, distilled water resulting in dilutions of

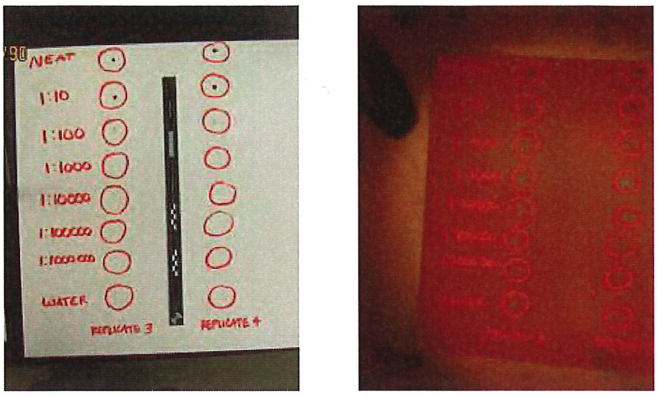

Figure 1

Organization of blood dilutions showing luminescence of the 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 dilutions on the whiteboard, replicates 3 and 4. The circle designated for water of replicate 3 also shows luminescence caused by a bubble of the working solution reacting with hydrogen peroxide. This reaction occurred in the presence of excess working solution, further proving the necessity of lightly spraying the surface from a distance.

1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000. Before applying the Hemascein, each surface was observed through an orange barrier using an alternate light source (Spex Handscope HS-100-12F, Horiba Jobin Yvon, Edison, NJ) set to CSS-white. One drop (30 microliters ± 1 microliter) of each dilution was deposited onto a designated area on each surface. The dilutions were allowed to dry at room temperature for 24 hours.

Four additional simulation surfaces were prepared by applying neat blood to the sole of a shoe and the left hand of one of the researchers. The hand and shoe were then pressed against the linoleum, wood paneling, a mirror, and a carpet. The hand and shoe received a fresh application of blood prior to each transfer stain. These surfaces were then washed with distilled water so that blood was not visible to the unaided eye and left to dry at room temperature for 24 hours.

After 24 hours, working Hemascein solution was sprayed onto each surface. Then each surface was sprayed with H_2O_2 and viewed using the alternate light source and orange goggles. Reactions were recorded and photographed for up to five minutes with three applications of Hemascein applied at intervals of approximately 60 seconds. This experiment was replicated four times and controls (water) were used.

Results

Linoleum

Four dilutions on the linoleum reacted with Hemascein: the 1:100, 1:1,000, 1:10,000, and 1:100,000 dilutions (Table 1). The first application of Hemascein and H_2O_2 on the linoleum resulted in luminescence within 60 seconds at dilutions of 1:1,000 (1/4 replicates), 1:10,000 (2/4 replicates), and 1:100,000 (3/4 replicates). After 60 seconds, the 1:1,000 dilution resulted in additional luminescence in two replicates. The second application on the linoleum resulted in new luminescence after 60 seconds in the 1:100 (2/4 replicates), 1:1,000 (1/4 replicates), 1:10,000 (2/4 replicates), and 1:100,000 (1/4 replicates) dilutions. No new luminescence was observed following the third application (Table 1).

Wood Paneling

Four dilutions on the wood paneling reacted with Hemascein: the 1:100, 1:1,000, 1:10,000, and 1:100,000 dilutions (Table 1). The first application of Hemascein and H_2O_2 on the wood paneling resulted in luminescence within 60 seconds at dilutions of 1:100 (1/4 replicates) and 1:1,000 (1/4 replicates). The second application on the wood paneling resulted in new luminescence within 60 seconds of the 1:1,000 (2/4 replicates), 1:10,000 (1/4 replicates), and 1:100,000 (1/4 replicates) dilutions. The second application on the wood paneling resulted in luminescence after 60 seconds of the 1:100,000 dilution (1/4 replicates). Only refreshed luminescence was observed following the third application (Table 1).

Whiteboard

Four dilutions on the whiteboard reacted with Hemascein: the 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 dilutions (Table 1). The first application of Hemascein and H_2O_2 on the whiteboard resulted in luminescence within 60 seconds at dilutions of 1:100,000 (2/4 replicates) and 1:1,000,000 (2/4 replicates). The second application on the whiteboard resulted in new luminescence within 60 seconds of the 1:1,000 (2/4 replicates) and 1:10,000 (2/4 replicates) dilutions. The second application on the whiteboard resulted in new luminescence after 60 seconds of the 1:1,000 (2/4 replicates), 1:10,000 (2/4 replicates), 1:100,000 (2/4 replicates) and 1:1,000,000 (2/4 replicates) dilutions. Only refreshed luminescence was observed following the third application (Table 1).

		Blood Concentration							
		Neat	1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	Water
Application 1	Linoleum				○□□	○○	○○○		
	Wood Paneling			○	○				
	Whiteboard						○○	○○	
	Porcelain Tile								
	Carpet								
Application 2	Linoleum			□□	□■■■	□□■■	□■■■		
	Wood Paneling			■	○○■	○	○□		
	Whiteboard				○○□□	○○□□	□□■■	□□■■	
	Porcelain Tile			□	□□				
	Carpet	○○○○	○○○○	○○○○	○	○			○
Application 3	Linoleum			■■	■■■■	■■■■	■■■■		
	Wood Paneling			■	■■■	■	■■		
	Whiteboard				■■■■	■■■■	■■■■	■■■■	
	Porcelain Tile			■	■■	□□□□	□□□	□	□
	Carpet	■■■■	■■■■	■■■■	■	■			■

○ Indicates new luminescence within 60 seconds of application.
□ Indicates new luminescence after more than 60 seconds following application.
■ Indicates refreshed luminescence.

Table 1

Luminescence of a range of blood (30 microliters ± 1 microliter) concentrations and negative control (water) following three applications of Hemascein on contrasting surfaces. Total observation time for each stain was approximately five minutes.

Porcelain Tile

Five dilutions and the negative control on the porcelain tile reacted with Hemascein: the 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 dilutions (Table 1). The first application of Hemascein and H_2O_2 on the porcelain tile did not result in luminescence. The second application on the porcelain tile resulted in luminescence after 60 seconds of the 1:100 dilution (1/4 replicates) and the 1:1,000 dilution (2/4 replicates). The third application resulted in new luminescence of the 1:10,000 (4/4 replicates), 1:100,000 (3/4 replicates), 1:1,000,000 (1/4 replicates) dilutions, and the negative control.

Carpet

Five dilutions and the negative control on the carpet reacted with Hemascein: the neat, 1:10, 1:100, 1:1,000, and 1:10,000 dilutions (Table 1). The first application of Hemascein and H_2O_2 on the carpet resulted in no luminescence. The second application on the carpet resulted in luminescence within 60 seconds of the neat (4/4 replicates), 1:10 (4/4 replicates), 1:100 (4/4 replicates), 1:1,000 (1/4 replicates), and 1:10,000 (1/4 replicates) dilutions, and water. The third application resulted in refreshed luminescence only.

Additional Simulation Surfaces

Hemascein reacted with the four additional surfaces prepared with hand and shoe transfers that were cleaned with distilled water. Distinct handprints and footmarks were clearly visible after one application of Hemascein and H_2O_2 (Figures 2, 3).

Discussion

Hemascein reacted with most blood dilutions following three applications within five minutes. It had the greatest degree of reliability within a dilution range of 1:1,000 to 1:100,000: reactions occurred 100% of the time on linoleum and whiteboard, 75% of the time on porcelain tile, 50% of the time on wood paneling, and 17% of the time on carpet. This range of reactivity is comparable to that of fluorescein [9, 10], luminol [2], and Bluestar [2], all of which are associated with luminescence within dilutions of 1:100,000. More generally, Hemascein reacted with the greatest degree of reliability on hard, flat, light-colored surfaces (lineoleum, whiteboard, wood paneling). Hemascein was apparently more sensitive than fluorescein, luminol, and Bluestar on the whiteboard; it reacted with 100% reliability at a concentration of 1:1,000,000.

The observation that Hemascein was 100% reliable within the low dilution range (neat, 1:10, 1:100, 1:1,000) on carpet was unexpected. The low level of reliability of Hemascein on carpet with dilute blood drops was also unexpected; it is possible that this reliability would have increased with an observation time greater than five minutes. We believe that the relatively small amount (30 microliters ± 1 microliter) of dropped blood on each surface was an effective test for the reliability and sensitivity of Hemascein. Generally, the least reliable reactions were associated with dilute blood on dark surfaces. In contrast, the surfaces prepared with transfer stains from a hand and the sole of a shoe reacted immediately and clearly. These transfer stains remained detailed, with the handprints showing ridge detail and the shoeprints showing apparent tread patterns, both of which could be useful for identification. These transfer stains better represent stains observed in the field, whereas the blood drops represent a test of the ability of Hemascein to react with small amounts of dilute blood.

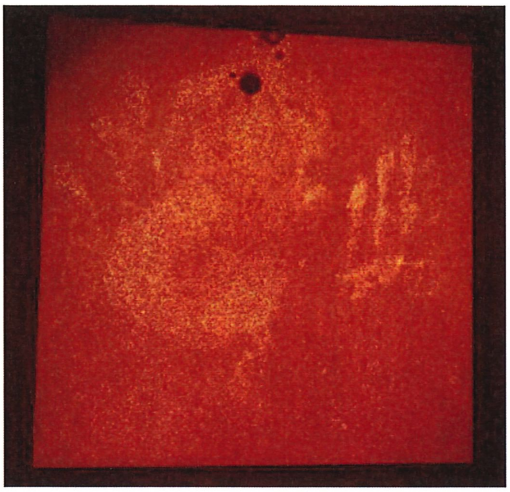

Figure 2

Latent transfer stains on linoleum. Once the neat blood was transferred, the surface was cleaned with distilled water and left to dry for 24 hours at room temperature. (The dark region at the top and center of the linoleum was a manufacturing defect and is unrelated to the process of this experiment.)

Figure 3

Latent transfer stain on carpet. Once the neat blood was transferred to the carpet, it was allowed to dry for 24 hours at room temperature.

The spraying technique and false positives must be considered when using Hemascein, because it had an important effect on discovery. We found that the spray worked best when applied lightly above the stain so the solution could drift down onto the surface from a height of approximately 30 centimeters (approximately 12 inches). This spray technique enhanced discovery and decreased background luminescence. It was also important to wait for the reaction to occur. Unlike luminol or Bluestar, Hemascein does not react to its full capacity instantly. A few seconds are required to allow the reaction to complete. Once the reaction is complete, the luminescence lasts long enough to take photographs without having to reapply the reagents. Also, additional sprays using the same height and technique were used to refresh the luminescent reaction (Table 1).

It was also noted that Hemascein reacted with water on the carpet after a second and third application and with water on the porcelain tile after the third application. One possible explanation is that the accumulation of the working solution over multiple applications reacted with the hydrogen peroxide, resulting in a false postive. In other words, we observed concentrated islands of background fluorescence. This is supported by the fact that these false reactions were only noted after multiple applications were made.

Another consideration is the effect of Hemascein on subsequent DNA analysis. Fluorescein [11], luminol [12], and BlueStar [7] have been assessed for their effect on the use of latent blood as a source of DNA, and each of these discovery techniques can be used without compromising the analysis of autosomal DNA. Hemascein must also be explored experimentally for its effect on nucleotide sequence analysis. It can be inferred that Hemascein will not affect the analysis of nuclear DNA because it is a fluorescein-based technique and fluorescein does not compromise the forensic use of short tandem repeats [7]. Past experiences in the field have produced viable DNA results after bloodstains have been detected using Hemascein [6]. However, the effect of Hemascein on the analysis of autosomal DNA has not been published and should be considered before Hemascein can become a regularly employed discovery technique.

Conclusion

The purpose of this paper is not to determine whether Hemascein is a "better" tool for the discovery of latent bloodstains. Rather, the current work aims ultimately to contribute

to the development of a robust and effective toolkit for the crime scene investigator and bloodstain pattern analyst. Several discovery techniques are available to choose from, including luminol, Bluestar Forensic, fluorescein, and Hemascein. All of these chemistries have strengths and weaknesses. The current work shows that Hemascein can be used reliably to discover latent blood on hard, flat, light-colored surfaces up to a dilution of 1:100,000. It also has the sensitivity to luminesce with latent blood at a concentration of 1:1,000,000. On charcoal-colored carpet, Hemascein possesses the ability to detect relatively concentrated blood (neat, 1:10, 1:100, 1:1,000) with a high level of reliability. These factors, along with the capabilities of fluorescein, luminol, and Bluestar, should be considered when developing a strategy for the discovery of latent bloodstains at a crime scene.

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