

Determining the Sensitivity and Reliability of HemaScein™

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By

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Dedication

This thesis is dedicated to Dr. David O. Carter, Sergeant Larry Barksdale, Kaitlin M. Leslie, my parents Tony and Shelly Lowis, my sister Brooke Lowis, and my boyfriend Joshua K. Hoenshell. Without these individuals, it would never have come to fruition.

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Abstract

Bloodstain analysis has been regarded as an important investigative tool in the field of forensic science as early as the mid-1800's. As such, a variety of tests and methods that detect bloodstains and to link them to specific individuals have evolved into the tests and methods in use today. These include methods of detecting latent bloodstains, or bloodstains that are for one reason or another no longer visible to the naked eye. Some of the most common tests for the detection of latent bloodstains include luminol, Bluestar™, Polilight®, and Fluorescein.

HemaScein™ is a new fluorescein-based method developed by Abacus Diagnostics. It is a spray that is applied to a surface suspected of containing a possible bloodstain. It is based on the chemiluminescent reaction between fluorescein and the heme in blood. At this point in time, few studies have assessed the sensitivity and reliability of HemaScein™. This experiment attempted to address this issue. A range of dilutions of human blood (Neat, 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, 1:1,000,000, and water) were made and deposited on a variety of surfaces (wood paneling, carpet, linoleum, whiteboard, and porcelain tile) and then tested with HemaScein™.

Based on the results, it was determined that HemaScein™ reacted with the greatest reliability on blood dilution ranges of 1:1,000 to 1:100,000, and was comparable to other common latent bloodstain detection methods. It was found to be most sensitive and reliable on light, smooth, flat surfaces. It was also found to be extremely reliable and sensitive to neat and 1:10 dilutions of blood on dark, absorbant surfaces. Its benefits include the lack of safety issues associated with its use. Its drawbacks include a high degree of background staining if sprayed improperly. Further testing to assess the effect of HemaScein™ on subsequent DNA analysis is recommended.

Introduction

History of Bloodstains

The importance of the identification and examination of blood as it relates to crime scene investigation has long been regarded as important. Records as early as 1220 exist associating the presence of blood as evidence of criminal activity in the Germanic Common Law, known as the *Sachsenspiegel*. Similar allusions to bloodstains occur in everything from Shakespeare to Finnish art. Sir Arthur Conan Doyle even introduced the character for which he became famous, Sherlock Holmes, in a novel titled *A Study in Scarlet*—a novel with a plot line that centered around the identification of bloodstains (Bevel, 2002). As a result of this recognized connection between bloodstain evidence and crime investigation, the development of bloodstain discovery techniques have been recorded as early as 1853, when Ludwig Teichmann developed a microscopic crystal based test for the presence of hemoglobin. This quickly opened the door to inventions with similar purposes by the Dutch and Germans, so much so that as early as 1895 Eduard Piotrowski wrote and published his book *Concerning the Origin, Shape, Direction and Distribution of the Bloodstains Following Head Wounds Caused by Blows* (Newton, 2008). This book, supported by extensive experimentation, detailed how to determine the directionality of bloodstains, as well as the different types of bloodstain patterns that occur as a result of different actions, including weapon castoff and stains associated with respiration (Bevel, 2002). By 1901, Professor A. Florence had classified bloodstains by the events that led to their formation, including dripping, grazing, splashing, and spurting (Bevel, 2002).

As the scope of bloodstain detection grew, the need for tests that could distinguish human blood from the blood of other animals arose. The 1900 discovery by Karl Landsteiner of human blood groups helped this cause, and Landsteiner was awarded the Nobel prize in 1930 for his

discovery. His discovery led to an entirely new approach to blood analysis, and eventually spawned a variety of blood group systems, including the Lewis, Kell, Duffy, and Kidd blood group systems. However, these systems were somewhat negated in the 1960s as Maurice Muller used the Ouchterlony antibody-antigen diffusion test to differentiate between the blood of species. In 1915, Leone Lattes developed a saline solution that was able to rehydrate dried bloodstains without interfering with the blood type analysis (Newton, 2008).

As methods and systems attempting to enhance the field of bloodstain detection continued to increase, Culliford wrote and published *The Examination and Typing of Bloodstains in the Crime Laboratory*, which helped to validate bloodstain discovery methods. In 1955, bloodstain analysis was used in court for the first time. Dr. Paul Kirk was called in to testify for the defense in the case of *The State of Ohio vs. Sam Sheppard*. Dr. Kirk was able to present the court with the relative positions of the victim and the assailant based on his analysis. He also testified that the bloodstain patterns that resulted from the beating of the victim were caused by a left-handed individual. Sam Sheppard was right-handed (Newton, 2008). Identification and collection of blood evidence became even more important in 1984 when Sir Alec Jeffreys developed the first DNA profiling test, a test that was used in 1986 to identify a murderer in England. In 1987, the admissibility of DNA was challenged for the first time in the court case *New York vs. Castro*. This case initiated an overhauling of forensic science, one that required accreditation, standardization, and quality control. This overhaul and its effects are still felt in the field today (Inman, 2001), as research regarding analysis of bloodstains and bloodstain detection continue. This continuing research can be found in the Forensic Science literature accessed through the International Association of Bloodstain Pattern Analysts, as well as through other Forensic Science organizations.

Background Information

A bloodstain is formed when blood is deposited on a surface (SWGSTAIN 2009). The analysis of bloodstain patterns can result in crime scene reconstruction relevant to the spatial origin of the blood, cause of the bloodletting event, and movement of the persons involved in the bloodletting event (Tobe et al. 2007). Blood is also important in regards to its source. Often, the detection of blood is important so that it can be sent in for subsequent DNA testing. Yet, it is not uncommon for bloodstains to decompose over time, be cleaned up from a crime scene or be otherwise invisible to the unaided eye. The ability to identify and recover latent bloodstains such as these is often important to bloodstain pattern analysis. From this need, the development and delivery of bloodstain discovery techniques has been a recent emphasis (e.g. Howard and Nessian 2010, Middlestead and Thornton 2010).

Luminol is arguably the most popular bloodstain discovery chemical. Its use relies on a chemiluminescent reaction catalyzed by iron in the blood (Dilbeck 2006). It has a recorded sensitivity of at least 1:100,000 (Tobe et al. 2007), is commercially available and easily prepared at a crime scene. It has also been shown that luminol does not compromise the integrity of bloodstains or the possibility of yielding STR results (Jakovich 2007, Tobe et al. 2007). However, luminol requires almost total darkness and luminesces for a short time only, making photography difficult. It also has an incredibly short shelf life, and must be prepared and used at the scene. Further complicating this situation, 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 modify its shape. Luminol has also been observed to luminesce in the presence of fresh bleach, strong metal ions, and strong peroxidases, such as horseradish (Dilbeck 2006). Luminol also has associated health considerations. It has been known to cause chemical burns or skin rashes and is

a hazard to anyone who comes into direct contact with it. This introduces increased costs associated with scene clean up or compensation for damaged products. Several law enforcement agencies have had to pay extreme liability claims resulting from the use of luminol (Barksdale, Personal Communication, 2011). Additionally, repeated spraying can ruin the integrity of bloodstains.

Bluestar™, like luminol, is a liquid that is sprayed onto surfaces. Also like luminol, Bluestar™ exploits peroxidase-like activity of blood as a catalyst to produce chemiluminescence. Its luminescence is brighter and longer than that of luminol (Young 2006) and it has a shelf life of several days. It has also shown to be more successful at detecting latent blood that has been cleaned with bleach (Young 2006). As a Luminol based product, Bluestar™ has been associated with the aforementioned health hazards and can also ruin the integrity of bloodstains.

Alternative Light sources are also used to discover latent blood stains. Polilight®, unlike the previous three, is not a spray, but a light source that produces very narrow and intense bands of light from 310 to 650 nanometers (nm), with 415 nm producing the best results when looking for bloodstains. It has a recorded sensitivity of 1:1000 (Vandenberg and van Oorschot 2006) and is rapid and easy to use. Furthermore, its use concerning bloodstains does not appear to be compromised by contaminants or environmental exposure (Vandenberg and van Oorschot 2006). The advantages of alternative light sources can be seen when they are used on dark substrates, as the lights make bloodstains appear darker than their substrates.

A newer discovery technique is HemaScein™, which is a fluorescein based chemical. Fluorescein is a chemical compound that is reduced to fluorescein when it is combined with water. Fluorescein is then applied to a presumptive bloodstain, followed by an application of

hydrogen peroxide (H₂O₂). The reaction between H₂O₂ and fluorescein is catalyzed by heme present in blood (Figure 1).

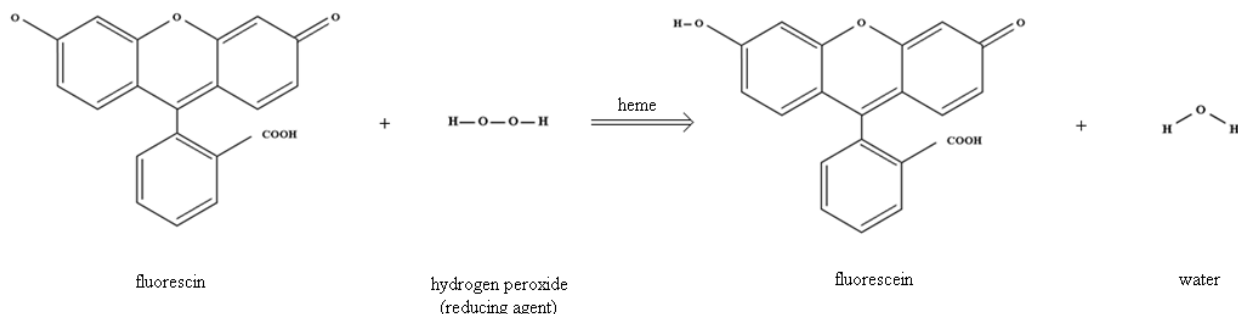


Figure 1. The hydrogen peroxide donates an electron to fluorescein, generating fluorescein and water. Fluorescein then luminesces when excited by a light source within the range of 415 nanometers and 480 nanometers. It has an observed sensitivity of 1:105,000 (Cheeseman, 1995) to 1:1,000,000 (Barksdale, 2010).

“Unpublished reports indicate that fluorescein detected blood on a shirt that was worn by a homicide victim and was tested eight years after the homicide. The swabbing of the reaction areas produced a full DNA profile that was not consistent with the victim’s. HemaScein has been used at a crime scene to detect comparable latent prints developed in blood on a vertical surface. Eighteen months later, the latent prints still reacted to light, were photographed, and were identified.” (Barksdale, Personal Communication, 2011) While HemaScein has been shown to be a valuable tool for bloodstain detection, at present the reliability and sensitivity of HemaScein™ is 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 (porcelain, vinyl paneling, wood paneling, carpet) in a range of concentrations (neat to 1:1,000,000).

Materials and Methods

Blood

Blood was drawn intravenously by a phlebotomist into sterile tubes without preservatives and anticoagulants.

Surfaces

Experimental surfaces include an Olefin Carpet Door Mat (Shaw, Charcoal colored, Product # 17357, 45.72 cm x 68.58 cm), White Board (Decorative Panels International, Bluelinx Paneling, Product # 709-106), Wood Paneling (Traffic Master, Redwood colored, Product # 361-057, Run #25.01.2010, 30.48 cm x 30.48 cm), Porcelain Tile (US Ceramic Tile Co., black colored, Product # U759-44-1M, 10.795 cm x 10.795 cm), Vinyl Paneling (Decorative Panels International, white colored, Product # A346-428, 60.96 cm x 60.96 cm).

Experimental Design

Each surface 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 30 microliters was dropped onto each surface in the area designated for 'neat' blood. Neat blood was diluted with sterile, distilled water resulting in dilutions of 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000. Before applying the Hemascein™, each surface was observed with an alternative light source (ALS) and orange goggles for background staining that might interfere with the bloodstain discovery. One drop of each dilution was deposited onto designated area on each surface. The dilutions were allowed to dry for twenty four hours.

Four additional simulation surfaces were prepared by rubbing undiluted neat blood onto the sole of a shoe and the left hand of one of the researchers. These were swiped onto linoleum, wood paneling, a mirror, and a carpet. A fresh application of blood was applied to the hand and shoe for each surface. These surfaces were then washed with distilled water so that blood was not visible to the unaided eye, and left to dry. Working Hemoscein™ solution was sprayed onto each surface. Then, one at a time, each surface was sprayed with H₂O₂ and viewed with the ALS and orange goggles. Reactions were recorded and photographed for up to five minutes with three applications of Hemoscein™ applied at intervals of approximately 60 seconds. This experiment was replicated four times.

Results

Linoleum

Four dilutions on the linoleum reacted with Hemascein™. These included 1:100, 1:1,000, 1:10,000, and 1:100,000 (Table 1). The first application of Hemascein™ and hydrogen peroxide 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 2 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). No new luminescence was observed following the third application (Table 1).



Figure 1. A crime scene simulation using undiluted blood on linoleum. Once the blood was dry, the surface was washed with tap water and let to dry. The dark region at the top and center of the linoleum was a manufacturing defect and unrelated to the process of this experiment.

Wood Paneling

Four dilutions on the wood paneling reacted with Hemascein™. These included 1:100, 1:1,000, 1:10,000, and 1:100,000 (Table 1). The first application of Hemascein™ and hydrogen peroxide on the wood paneling resulted in luminescence within 60 seconds at dilutions of 1:100 (1/4 replicates) and the 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). The second application on the wood paneling resulted in luminescence after 60 seconds of the 1:100,000 dilution (1/4 replicates). No new luminescence was observed following the third application (Table 1).

White Board

Four dilutions on the white board reacted with Hemascein™. These included 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 (Table 1). The first application of Hemascein™ and hydrogen peroxide on the white board resulted in luminescence within 60 seconds at dilutions of 1:100,000 (2/4 replicates) and the 1:1,000,000 (2/4 replicates). The second application on the white board resulted in new luminescence within 60 seconds of the 1:1,000 (2/4 replicates) and 1:10,000 (2/4 replicates). The second application on the white board resulted in new luminescence after 60 seconds of the 1:1,000 (2/4 replicates), 1:10,000 (2/4 replicates), 1:100,000 dilution (2/4 replicates) and the 1:1,000,000 dilution (2/4 replicates).

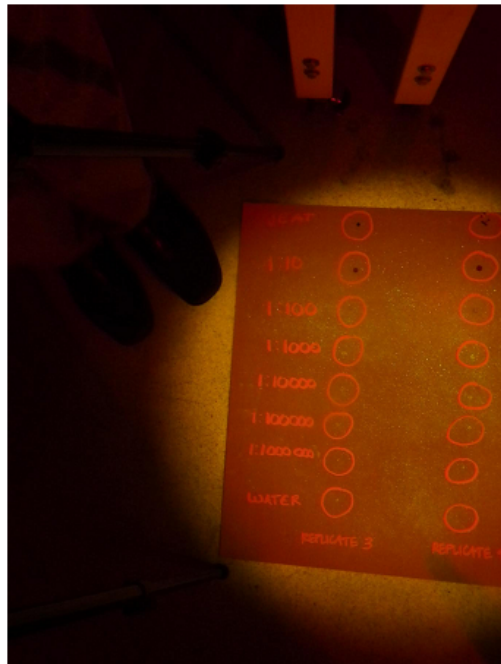
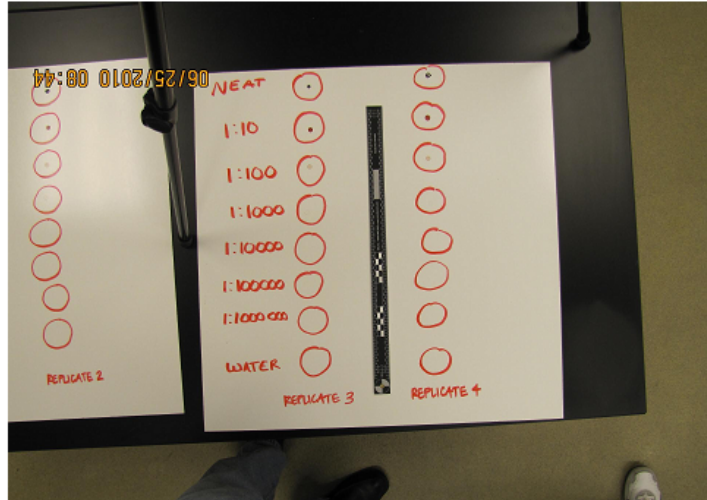


Figure 2. Luminescence of the 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 dilutions of the white board, replicates 3 and 4. The circle designated for water of replicate 3 also shows luminescence cause by a bubble of the working solution reacting with hydrogen peroxide that was later applied. This reaction occurred in excess presence of working solution, further proving the necessity of lightly spraying the surface from a distance.

No new luminescence was observed following the third application (Table 1).

Porcelain

Five dilutions and the negative control on the porcelain reacted with Hemoscein™. These dilutions included 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 (Table 1). The first application of Hemoscein™ and hydrogen peroxide on the porcelain did not result in luminescence. The second application on the porcelain 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 dilution (4/4 replicates), the 1:100,000 dilution (3/4 replicates), the 1:1,000,000 dilution (1/4 replicates) and the water.

Carpet

Five dilutions and the negative control on the carpet reacted with Hemoscein™. These included the neat, 1:10, 1:100, 1:1,000, 1:10,000 (Table 1). The first application of Hemoscein™ and hydrogen peroxide 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 dilution (4/4 replicates), and 1:100 (4/4 replicates), the 1:1,000 dilution (1/4 replicates), the 1:10,000 (1/4 replicates) and water. The third application resulted in no new luminescence.

Additional Simulation surfaces

Hemoscein™ also reacted with the four additional surfaces prepared by making hand and footmarks with undiluted blood that were washed off with distilled water. Distinct handprints and footmarks were clearly visible after one application of Hemoscein™ and H₂O₂ and continued to be visible after additional sprays.



Figure 3. A crime scene simulation on a sample of the carpet. This was prepared with undiluted blood.

Table 1. Luminescence of a range of blood (30 microliters) concentrations and negative control (water) following three applications of HemaScein™ on contrasting surfaces where ○ indicates new luminescence within 60 seconds of application, □ indicates new luminescence greater than 60 seconds following application, and ■ indicates refreshed luminescence.

		Blood concentration							
		neat	1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	water
Application 1	Linoleum				○□□	○○	○○○		
	Paneling			○	○				
	White board						○○	○○	
	Porcelain								
	Carpet								
Application 2	Linoleum			□□	□■■■	□□■■	□■■■		
	Paneling			■	○○■	○	○□		
	White board				○○□□	○○□□	□□■■	□□■■	
	Porcelain			□	□□				
	Carpet	○○○○	○○○○	○○○○	○	○			○
Application 3	Linoleum			■■	■■■■	■■■■	■■■■		
	Paneling			■	■■■	■	■■		
	White board				■■■■	■■■■	■■■■	■■■■	
	Porcelain			■	■■	□□□□	□□□	□	□
	Carpet	■■■■	■■■■	■■■■	■	■			■

Discussion

HemaScein™ reacted with most dilutions following three consecutive applications. 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 vinyl tile and white board, 75% of the time on porcelain, 50% of the time on paneling, and 17% of the time on carpet. This range was comparable to that of fluorescein (Cheeseman and DiMeo 1995, Cheeseman 1999), luminol (Tobe et al. 2007) and Bluestar™ (Tobe et al. 2007); 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 (vinyl tile, white board, wood paneling). HemaScein was apparently more sensitive than fluorescein, luminol and Bluestar™ on the white board, as 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 5 minutes. We believe that the relatively small amount (30 microliters) 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 latent transfer stains from a hand and the sole of a shoe reacted immediately and clearly. These swipes remained detailed, with the hand swipes showing ridge detail and the shoe swipes showing apparent tread patterns; both of which could be useful for identification. These transfer stains are likely to better

represent stains observed in the field, whereas the blood drops represent a rigorous test of the ability of HemaScein™ to react with small amounts of dilute blood.

Spraying technique must also be considered when using HemaScein™, as it had an important effect on discovery. We found that the spray worked best when applied lightly above the stain so the solution can 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 (see Table 1).

Another consideration is the effect of HemaScein™ on subsequent DNA analysis. Fluorescein (Budowle et al. 2000), luminol (Gross et al. 1999), and BlueStar (Jakovich 2007) 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. However, HemaScein™ must also be explored for its affect on nucleotide sequence analysis. It can be inferred that HemaScein™ will not affect the analysis of nuclear DNA since it is a fluorescein-based technique and fluorescein does not compromise the forensic use of short tandem repeats (Jakovich 2007). Past experiences in the field have produced viable DNA results after a bloodstain was detected using HemaScein™ (Barksdale, Personal Communication, 2011). 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.

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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