

# **Detecting Burnt Bloodstain Samples with Light-Emitting Blood Enhancement Reagents**

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## **INTRODUCTION**

When criminals deliberately set fires or explosions to cover their activities, the resulting crime scenes are very difficult to process. Crime scene investigators are faced with the difficult task of locating, recognizing, and identifying biological evidence amongst the debris resulting from the fire or explosion (1). Biological and non-biological stains can appear similar when charred by the high temperatures associated with a fire or an explosion (2,3). Rapid and sensitive screening tests are required to assist the crime scene investigators with the task of selecting relevant biological material for subsequent DNA typing analysis.

Forensic scientists use a variety of blood screening tests to detect the presence of blood at crime scenes (4,5). Typically, only the stains which yield a positive result with the blood screening tests are collected for subsequent DNA analysis. However, when the perpetrator had attempted to clean or remove blood evidence from a crime scene, sensitive light-emitting blood enhancement reagents can be employed to locate residual quantities of blood (6,7).

Luminol is a very sensitive blood enhancement reagent which is widely used to detect trace quantities of blood at crime scenes (8,9). Another blood enhancement reagent, Bluestar®, uses a modified form of the luminol molecule to produce a very bright chemiluminescence-based blue-light emission (10,11). Both luminol and Bluestar® reagents have a short shelf life and must be prepared just before use. Furthermore, the crime scene must be darkened in order to see the short-lived light emissions. Hemascein™ is a fluorescein-based product which emits a fluorescence-based green light when excited with an intense blue-light source (12). One practical advantage of this reagent is the long shelf life of the working solution, several months when stored at refrigerator temperatures. Fluorescein, a blood enhancement reagent related to Hemascein™, was shown to outperform both Bluestar® and luminol when used to detect burnt bloodstain patterns (3).

A quantitative comparison of three different light-emitting blood enhancement reagents: luminol, Bluestar®, and Hemascein™, was performed in this study in order to evaluate their ability to detect bloodstains which had been exposed to the heat and smoke of a fire.

## **MATERIALS AND METHODS**

### **Blood Samples:**

- Canine blood was chosen for this study for health and safety reasons, and for its similarity to human blood in red blood cell count and hemoglobin concentration (13). Canine blood in Vacutainer® tubes with EDTA as a preservative was obtained from the Cheney Veterinary Clinic.
- Serial dilutions of canine blood ranging from 1:10 to 1:10,000 were prepared using distilled water.

- Control bloodstain smears of approximately  $1\text{ cm}^2$  were prepared on glass microscope slides using  $5\text{ }\mu\text{l}$  of a 1:10 dilution of liquid blood.
- Burnt bloodstains were prepared using  $5\text{ }\mu\text{l}$  of the 1:10 dilution of liquid blood and heat treated by direct exposure to the flame of an alcohol fire for 1, 3, or 5 minutes (Figure 1). Bloodstains were subjected to temperatures ranging from 400-600 °C.



Figures 1a and 1b. Preparation of Burnt Bloodstains: glass microscope slides with bloodstain smears were suspended face-down over a 10 cm diameter glass Petri dish filled with 10 ml of absolute ethanol for the burn experiment.

#### **Blood Enhancement Reagent Preparation:**

- Luminol was prepared according to the Grodsky formulation, modified with the addition of sodium hydroxide to a final concentration of 25 mM (10).
- Bluestar® was prepared fresh by adding the two-prepackaged tablets to 125 ml of distilled water as per the manufacturer's instructions (11).
- Hemascein™ stock and working solutions were prepared according to the manufacturer's instruction (12). For the purpose of this study, a test buffer was prepared just before use by mixing one part of the Hemascein™ working solution with one part of 3% hydrogen peroxide

#### **Emission Spectroscopy:**

- A Bio-Rad VersaFluor™ fluorometer was used to record light emissions. The intensity of the light emissions in Relative Fluorescence Units [RFU] was recorded every 7 seconds over the 5 minute testing period.
- Light emissions greater than 20,000 RFUs exceeded the instrument's quantitation limit.
- For blood tests with luminol and Bluestar®, the instrument's excitation light source was blocked and a  $460 \pm 5\text{ nm}$  filter was used to measure light emissions.
- For blood tests with Hemascein™, a  $460 \pm 5\text{ nm}$  excitation filter and a  $520 \pm 5\text{ nm}$  emission filter were used.
- For the analysis of liquid blood samples, a  $25\text{ }\mu\text{l}$  aliquot of diluted blood was mixed with 2 ml of blood enhancement reagent in a UV-transparent plastic cuvette.
- For the analysis of control and burnt bloodstain samples, individual stains were collected from the glass slide using a cotton swab moistened with distilled water. The collected stain was placed in a UV-transparent plastic cuvette containing 2 ml of blood enhancement reagent and mixed by inversion.
- All blood tests were performed in triplicate and the results averaged.

## **RESULTS AND DISCUSSION**

Table 1. Summary of the maximum light emissions obtained for liquid blood, control bloodstains, and burnt bloodstains tested with luminol, Bluestar®, and Hemascein™

Sample & Test Conditions			Maximum Light Intensity (RFUs)		
Sample Type	Blood Dilution*	Burn Time	Luminol	Bluestar®	Hemascein™
Liquid Blood	1:800	--	20,000**	20,000**	15004
	1:8,000	--	18525	20,000**	5001
	1:80,000	--	4368	9364	503
	1:800,000	--	361	146	ND
Control Bloodstain	1:4,000	--	20,000**	20,000**	11635
Burnt Bloodstains	1:4,000	1 min	481	11202	3957
	1:4,000	3 min	ND	1128	2042
	1:4,000	5 min	ND	785	867

\*Dilution values for blood in the test buffers

\*\* Light intensity exceeded instrument's quantitation limit

ND = Not detected or below 100 RFU (the light intensity which would not be visible to the naked eye)

### Liquid Blood:

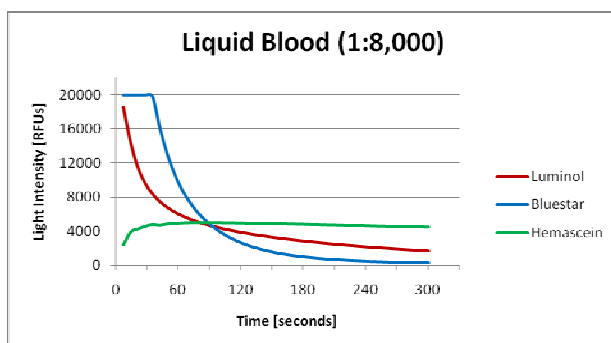


Figure 2. Intensity of light emissions obtained for liquid blood (1:8,000 dilution)

- Liquid blood treated with luminol showed high and rapidly decreasing light intensity readings during the initial 60 seconds, followed by a more gradual decrease of light intensity.
- Blood treated with Bluestar® gave the highest initial light intensity readings, decreasing rapidly during the initial 60 seconds, followed by a more gradual decrease of light intensity.
- Although Hemascein™ light emissions were not as intense as Bluestar® or luminol during the initial 90 seconds of the test, the reagent's light emissions were nearly constant over the entire 5 minute test period.

- Similar results were obtained with other dilutions of liquid blood. See Table 1 for maximum light intensity values with other liquid blood dilutions.

### Control Bloodstains:

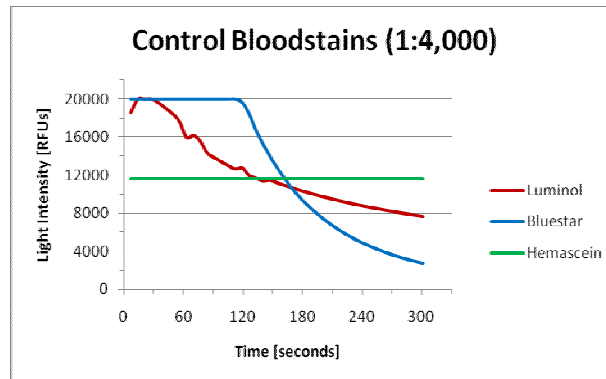


Figure 3. Intensity of the light emissions obtained for the control bloodstains (1:4,000 dilution)

- As observed with the liquid blood samples (Figure 2), the light emissions obtained for the control bloodstains with Bluestar® were more intense than the emissions obtained with luminol, with both reagents showing a decrease in light intensity beginning at approximately 30 seconds for luminol and 120 seconds for Bluestar®.
- The use of Hemascein™ resulted in light emissions which were not as intense as Bluestar® or luminol during the initial time period of the test, however, Hemascein™ light emissions were substantial and nearly constant over the entire 5 minute test period.

### Burnt bloodstains:

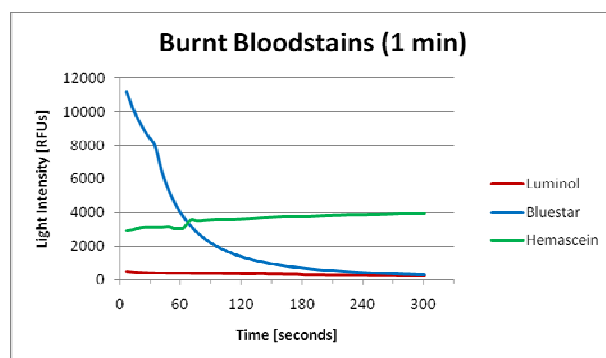


Figure 4. Intensity of light emissions obtained with the bloodstain samples burned for 1 minute.

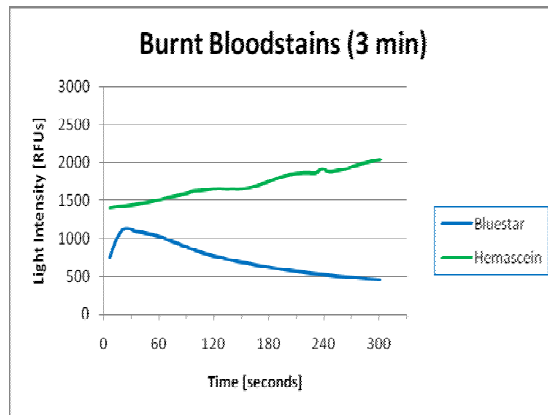


Figure 5. Intensity of light emissions obtained with the bloodstain samples burned for 3 minutes

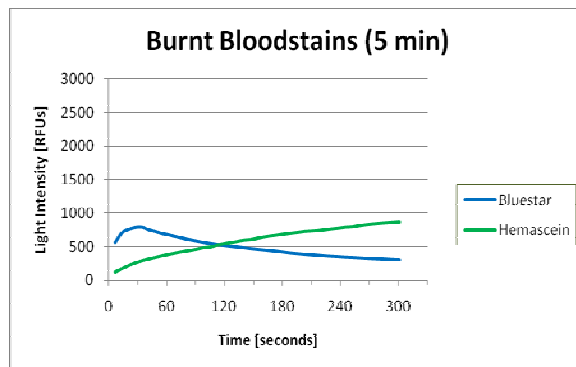


Figure 6. Intensity of light emissions obtained with the bloodstain samples burned for 5 minutes

- Luminol produced detectable light emissions only with bloodstains subjected to a 1 minute burn.
- Both Hemascein<sup>TM</sup> and Bluestar® had similar limits of detection, yielding significant light emissions with bloodstains subjected to a 1, 3, and 5 minute burn time.
- In contrast to the light emissions obtained with Bluestar®, Hemascein<sup>TM</sup> light emissions were nearly constant over the entire 5 minute test period.

## CONCLUSIONS

Our results show that the three blood enhancement reagents varied in their light emission characteristics, depending on whether the tested blood sample was a liquid, a stain, or a stain that had been exposed to very high temperatures of a fire.

Both luminol and Bluestar® produced an intense chemiluminescence-based light emission with liquid blood samples during the first minute of the test. This was followed by a rapid decay of light intensity. In contrast, Hemascein<sup>TM</sup> produced a nearly constant fluorescence-based light emission with

intensities that equaled or surpassed those of Bluestar® and luminol after the initial one minute time period.

Both Bluestar® and Hemascein™ outperformed luminol with respect to the detection of burnt bloodstains. Hemascein™ produced a nearly constant fluorescence-based light emission with intensities that equaled or surpassed those of Bluestar® after the initial one minute time period.

The above results suggest the potential benefit of using Hemascein™ to detect burnt blood at crime scenes. A study is in progress to optimize the Hemascein™ test conditions and analyze burnt bloodstains from simulated arson-homicide scenes.

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