BLOOD DETECTION

"A Comparison of Visual Enhancement Chemicals for the Recovery of Possible Blood Stains at the Crime Scene"
Luminol vs. BlueStar®

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The recovery and detection of possible blood stains at the crime scene can make or break a case, especially with the advent of and perfection of DNA testing. When a crime scene is cleaned up, this technology becomes even more important. Over the past two years we have conducted a study of Luminol and a new chemical BlueStar® Forensic this research delves into the specifics of each chemical, its makeup, use and results of the chemical testing on various blood stains of various dilutions to determine the sensitivity of each chemical.

From Luminol to BlueStar® Forensic : a brief history

The first experiments conducted with a view to using Luminol as a tool in forensic sciences were conducted in 1937 by **Specht**, who tested it out on a variety of bases such as the lawn, bricks, or stone soaked in blood. In 1939, Proesher & Moody tested Specht's composition on animal and human blood.

In 1951, **Grodsky** proposed a blend of powders made up of Luminol, Sodium Carbonate, and Sodium Perborate mixed with distilled water. This subsequently became the formula that is most commonly used by today's investigators to detect traces of blood at the scene of a crime.

However, the use of sodium carbonate produces a slow reaction in the oxidization process of hemoglobin. It therefore is not very luminous and of brief duration only. Moreover, once the reactive agents dissolve in the water, the life of the solution obtained is very short. This formula is very unstable and is toxic, due to the presence of Sodium Perborate.

In 1966, **Weber** proposed a composition made up of Luminol, sodium hydroxide or potassium hydroxide, hydrogen peroxide diluted in distilled water. The solution so obtained needs to be kept in a cool place away from direct light. Its lifespan is brief. The luminous reaction obtained by this composition can be photographed in total darkness, or filmed with a night-vision camera.

In 2000, Jean-Marc Lefebvre-Despeaux, president of Roc Import, charged Loic Blum, Ph.D., professor of bio-chemistry at the University Claude Bernard-Lyon and director of the enzymatic and biomolecular engineering laboratory (EMB2-UMR 5013 CNRS-UCBL) to find a new formula that would be Luminol-based and eliminate all those numerous inconveniences. As a result, Blum discovered this new formula that was subsequently called **BlueStar® Forensic** [1]. BlueStar® Forensic is a patented powerful new latent blood reagent.

Luminol:

How does Luminol react ?

Luminol (3-Aminophthalhydrazide) was synthesized for the first time in 1853. Its property to produce a chemo-luminescent reaction in basic solution in the presence of an oxidizing agent on contact with blood was first observed by Albrecht in 1928.
The main components capable of catalyzing this reaction for emitting light are the transition metals haem and peroxidase. Haem is a biochemical structure that forms an integral part of peroxidase. This structure (Figure #1) is equally present in hemoglobin. In this manner, the presence of hemoglobin – thus of blood – can be revealed by taking advantage of the ability of haem to catalyze the chemo-luminescent property of Luminol. In other words, a mix of Luminol + oxidizing agent + alkaline agent, when placed in contact with blood, will emit light [2].

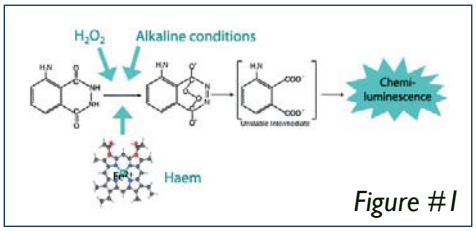


Figure #1

About the Authors

The authors of this article are M. Dawn Watkins and King C. Brown. Watkins is senior latent-print examiner/crime-scene investigator at the Palm Beach Gardens (Florida) Police Department. She has been in the forensic field for 32 years, holds a Masters Degree in Criminal Justice, and is an IAI Certified Latent Print Examiner and Senior Crime-Scene Analyst. Brown is the Crime-Scene supervisor at the West Palm Beach (Florida) Police department. He has been in the forensic field for 20 years, holds a Masters Degree in Criminal Justice, and is an IAI Certified Senior Crime-Scene Analyst and Forensic Photographer.

Figure #2

This light reaction reacts in the 441-452 Nm range emitting a blue/white light it is somewhat similar to that of a firefly. The reaction happens when oxidation occurs with the presence of hemoglobin, an extra Oxygen molecule jumps orbit and then returns to normal [2]. **Luminol is a "Luciferin"** (A Pigment Made By Organisms, Which are Capable of Bioluminescence). **Blood is a "Luciferase"** (An Enzyme Present in the Cells of Bioluminescent Organisms that Catalyzes the Oxidation of the Luciferin). Luminol is a presumptive test which gives off light instead of color.

Luminol has a long history is said to be non-destructive to blood, DNA, non-corrosive to the crime scene and highly sensitive 1:1,000,000. Luminol can detect the presence of blood and the presence of a cleanup and can detect patterns of removal. However, it does not react with enough resolution for fingerprint identification. (Figure #2)

Luminol does react and produce some false positives on the following types of surfaces:

- Plant Peroxidase-Plant Cells Which Act to Catalyze the Oxidation of a Substance By Peroxide.
- Chemical Oxidants-Chemicals Which Cause Oxidation
  - Certain Cleaning Chemicals
  - Metals
- Catalysts.
  - Specialized Material that Makes a Chemical Reaction Happen More Quickly (Bleach).

Luminol Formula:

- **25 Grams Sodium Carbonate.**
- **.5 Grams Luminol.**
- **3.5 Grams Sodium Perborate.**
- **500 ml. Distilled Water (500 ml. Solution).**

Luminol Reagent Preparation:

1. Add 4oz. of distilled water in a mixing bottle.
2. Add a 4oz. bottle of Luminol Pre-mixed to the water.
3. Mix the chemicals and the water thoroughly.
4. Decant off the liquid into a spray bottle, leaving the sediment in the bottom of the mixing bottle.
5. Always mix chemicals just prior to use.
6. Shelf Life is very limited to several hours, even after refrigeration.

Luminol Spraying Procedure:

1. Set spray bottle to the finest setting.
2. Prepare photographic equipment.
3. Darken the room, no light if possible.
4. Spray in a sweeping motion.
5. Immediately check for a reaction.
6. Photograph the reaction as specified.

Luminol Photography:

The Luminol reaction is very difficult to photograph and the higher the ISO film speed the shorter the exposure time for 35 mm or digital cameras.

Luminol Camera Settings 35 mm:

- 24 mm fast lens (f 1.14, 1.18, 2.0, 3.5)
- 400 ISO film or faster
- Camera on a tripod
- Cable Release
- Aperture f-stop 2.8 or wide opened
- Expose for 30sec. 45 sec. 60 sec. 90 sec. 120 sec.

Luminol Camera Settings Digital:

- 24 mm fast lens (f 1.14, 1.18, 2.0, 3.5)
- 800 ISO speed or faster
- Camera on a tripod
- Cable Release
- Aperture f-stop 2.8 or wide opened
- Expose for 15 sec. 30 sec. 45 sec. 60 sec. 90 sec. 120 sec. Check the view screen after each exposure for a properly exposed photograph.
- Complete Darkness is required.

BlueStar® Forensic:

BlueStar® Forensic (Figure #3) is a new reagent whose purpose is to reveal blood stains that have been washed out, wiped off or which are invisible to the naked eye. This product is intended for crime investigators. Based upon chemiluminescences, its unique formula qualifies it as the most effective blood revealer available on the market, for crime scene as well as forensic lab use. BlueStar® Forensic does not alter the DNA of the revealed blood which allows for its subsequent genotyping. Furthermore, it is non toxic, contrarily to the classic Luminol formula [2]. Some of the features of BlueStar® Forensic are:

- The extreme sensitivity of BlueStar® Forensic allows the naked eye to detect bloodstains down to 1/1000 dilutions, like minute traces or droplets that have been washed off, with or without detergent.
- BlueStar® Forensic has a stronger and longer-lasting luminescence that does not require total darkness to be visible. Good quality pictures can be obtained with an ordinary camera and film.
- With a little practice, BlueStar® Forensic makes it impossible to get confused between blood and false positives since the luminescence differs in color, intensity and duration.
- BlueStar® Forensic works as well on fresh blood as on very old or altered bloodstains, pure or diluted.
- BlueStar® Forensic is easier and safer to prepare. Neither powders nor liquids to be mixed, but only fast-dissolving tablets.
- BlueStar® Forensic can be sprayed over and over again on the same bloodstains for the same resulting luminescence each time.
- Before use, BlueStar® Forensic has a very long shelf-life and does not necessitate any special storage condition.
- Once mixed, BlueStar® Forensic is stable and will work for hours, or even several days.
- BlueStar® Forensic is not toxic, thanks to the absence of Sodium Perborate Tetra hydrate. Easily recycled. No residues left after drying. [2]

Figure #3

BlueStar® Forensic Sensitivity:

BlueStar® Forensic is more sensitive than other presumptive field tests for blood. Bloodstains treated with BlueStar® Forensic are visible over a 1/1000 dilution. When mixed with the catalyst hydrogen peroxide, and put in contact with the hem nucleus of blood hemoglobin, the Bluestar® Forensic oxidizes (enzymatic peroxydase activity) and emits an intense base (420-440 nanometer) chemiluminescence visible in the dark. Blood may be detected either pure or diluted, while fresh, or long after it has been dried, rinsed away, or cleaned away. [5] Its sensitivity is such that it will evidence blood in quantity smaller than the minimum required to perform D.N.A. typing. BlueStar® Forensic enables the detection of invisible or microscopic traces or blood droplets, particularly against dark backgrounds. The visualization of the stains does not depend on the size of the bloodstains, but only on the actual presence of blood [2].

BlueStar® Forensic produces a more intense and longer-lasting luminescence that does not need total darkness to be visible. BlueStar® Forensic can be sprayed several times on the same area, making observation and picture or movie taking easier. Evidential photographs can be taken with an ordinary camera and film, therefore suppressing the need for sophisticated equipment [2].

BlueStar® Forensic False Reactions:

Bluestar® Forensic may produce "false" reactions due to the presence of certain household detergents, chlorine, some paints, varnishes, copper, certain iron metabolizing plants such as lichens, thyme, and some tree mosses, and certain soils containing iron. However differences in intensity, color (emission specter), and duration allow for visual differentiation by experienced users. [5]

BlueStar® Forensic Photography:

The luminescence that occurs upon applying BlueStar® Forensic blood reagent lasts for several minutes and BlueStar® Forensic can be sprayed several times on the same area, making observation and picture taking easier. Evidential photographs can be taken with an ordinary camera and film, therefore suppressing the need for sophisticated equipment.

BlueStar® Forensic 35 mm Camera Settings:

- 24 mm lens
- Camera with a "Bulb" Setting.
- 400 ISO film
- Camera on a tripod
- Aperture f-stop 2.8
- Exposure for 30s [5]

BlueStar® Forensic Camera Settings Digital:

- 24 mm fast lens (f 1.14, 1.18, 2.0, 3.5)
- Camera with a "Bulb" Setting.
- 800 ISO speed or faster.
- Camera on a tripod.
- Cable Release
- Aperture f-stop 2.8 or wide opened.
- Expose for 15 sec. 30sec. 45 sec. 60 sec. 90 sec. 120 sec. Check the view screen after each exposure for a properly exposed photograph.

Procedure

All that is needed is a darkened room with diffused light. Natural diffused light is preferred to artificial light which gives yellowish or greenish pictures. A flash behind the photographer should be avoided because total darkness is difficult to obtain and is dangerous for the technician to move around. The technician should set the camera on a tripod preferably before treating the suspected area in order to avoid over spraying.

BlueStar® Forensic Chemical Preparation:

1. Add 4oz. of distilled water in a spray bottle.
2. Add a pair of BlueStar® Forensic tablets (Figure #4) to the water.
3. Allow 1-2 minutes for dissolution, stir gently. Do Not Shake.
4. Always mix chemicals just prior to use and do not use after 3 hours. [6]

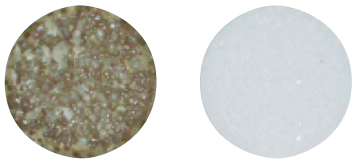


Figure #4

BlueStar® Forensic Spraying Procedure:

1. Set spray bottle to the finest setting.
2. Prepare photographic equipment.
3. Darken the room.
4. Spray in a sweeping motion.
5. Immediately check for a reaction.
6. Photograph the reaction as specified. [6]

Luminol vs. BlueStar® Sensitivity Testing Study

Procedure

Human blood was obtained via donation and refrigerated until use in the experiments. Distilled water was used to create dilutions (Figure #5) in the following ratios:

- 1:10
- 1:100
- 1:1000
- 1:5,000
- 1:10,000
- 1:50,000
- 1:100,000
- 1:1,000,000

Figure #5

This dilution was conducted as follows:

1) Add exactly .9ml (cc) of distilled water to each test tube.
2) One drop .01ml. of blood to test tube #1 (1:10).
3) Take exactly 0.1ml from test tube #1 and add it to test tube #2 (1:100).
4) Mix well and continue to the last dilution (1:1,000,000).
5) Label another tube 1:5,000.
6) Add 0.8ml of distilled water to this tube.
7) Take 0.2ml of the mixture in test tube #3 (1:1,000) and add it to the 1:5,000 dilution tube.
8) Label another tube 1:50,000.
9) Add 0.8ml of distilled water to this tube.
10) Take 0.2ml of the mixture from the 1:10,000 dilutions and add it to the 1:50,000 tubes. [4]

Blood stains were made on various samples, which would normally be encountered at a crime scene; these samples were allowed to dry and placed in

a normal interior environment at approximately 85°-95° F and an approximate relative humidity of 65%. Stains were made on the samples so a 10 day, 20 day and 30 day study could be accomplished. Samples included (Figure #6):

- Painted Dry Wall
- Wood (White Wood)
- Cloth
- Plywood (1/2 inch)
- Paper
- Carpet
- Vinyl Tile (Self Stick)

Day 10 Testing:

The samples of the substrates were prepared and placed on a plastic covered table for spraying with first Luminol and then new samples were placed on a cleaned table for spraying with BlueStar® the following are the results of the experiment after 10 days.

Painted Dry Wall, Cloth Material, White Paper, & Vinyl Floor Tile (Self Stick)

Luminol was applied to the substrate sample with positive results on all dilutions of 1:10, 1:100, 1:1,000, 1:5,000, 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000.

BlueStar® was applied to the substrate sample with positive results on all dilutions. The Dilutions of 1:10, 1:100, 1:1,000 & 1:5,000 were slightly stronger and brighter than the results from the 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000, however results were easily visible even on the dilution of 1:1,000,000.

BlueStar® was far superior to Luminol on this sample, because of the brightness of the reaction.

Wood (White Pine)

Luminol was applied to the substrate sample with positive results on the dilutions of 1:10, 1:100, 1:1,000 & 1:5,000. There were no results in the dilutions of 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000.

BlueStar® was applied to the substrate sample with positive results on all dilutions except for 1:1,000,000.

The BlueStar® out performed the Luminol in this sample and proved more sensitive and was much brighter than the Luminol test.

Figure #6

Plywood 1/2 Inch

Luminol was applied to the substrate sample with positive results on the dilutions of 1:10, 1:100, 1:1,000 & 1:5,000. There were no results in the dilutions of 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000

BlueStar® was applied to the substrate sample with positive results on the dilutions of 1:10, 1:100, 1:1,000, 1:5,000, 1:10,000 & 1:50,000. There were no results in the dilution, 1:100,000, 1:1,000,000.

The BlueStar® out performed the Luminol in this sample with positive results in the 1:10,000 & 1:50,000 dilutions and proved more sensitive and was much brighter than the Luminol test. It should also be noted that the Luminol and the BlueStar® reacted with the plywood and the blood dilutions.

Carpet

Luminol was applied to the substrate sample with positive results on all dilutions except for the dilutions of 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000.

BlueStar® was applied to the substrate sample with positive results on all dilutions.

The BlueStar® out performed the Luminol in this sample with positive results in the 1:10,000 & 1:50,000, 1:100,000 and 1:1,000,000 dilutions and proved more sensitive and was much brighter than the Luminol test.

20 Day Testing:

The samples of the substrates were prepared and placed on a plastic covered table for spraying with first Luminol and then new samples were placed on a cleaned table for spraying with BlueStar® the following are the results of the experiment after 20 days.

Painted Dry Wall, Cloth Material, White Paper, & Vinyl Floor Tile (Self Stick)

Luminol was applied to the substrate sample with positive results on all dilutions.

BlueStar® was applied to the substrate sample with positive results on all dilutions.

BlueStar® was far superior than Luminol on this sample, because of the brightness of the reaction.

Wood (White Pine)

Luminol was applied to the substrate sample with positive results on all dilutions except for 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000

BlueStar® was applied to the substrate sample with positive results on all dilutions except for 1:10,000, 1:50,000, 1:100,000 1:1,000,000.

The BlueStar® and Luminol reacted at the same levels of dilution; however the BlueStar® in this sample was much brighter than the Luminol test.

Plywood 1/2 Inch

Luminol was applied to the substrate sample with positive results on all dilutions except for the dilutions of 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000.

BlueStar® was applied to the substrate sample with positive results on all dilutions except for 1:10,000 & 1:50,000, 1:100,000, 1:1,000,000.

The BlueStar® and Luminol were even in the sample testing; however the BlueStar® was brighter. It should also be noted that the Luminol and the BlueStar® reacted with the plywood and the blood dilutions.

Carpet

Luminol was applied to the substrate sample with positive results on all the dilutions except for

1:10,000, 1:50,000, 1:100,000 and 1:1,000,000.

BlueStar® was applied to the substrate sample with positive results on all dilutions

The BlueStar® out performed the Luminol in this sample with positive results in the 1:10,000 & 1:50,000, 1:100,000 and 1:1,000,000 dilutions and proved more sensitive and was much brighter than the Luminol test.

30 Day Testing:

The samples of the substrates were prepared and placed on a plastic covered table for spraying with first Luminol and then new samples were placed on a cleaned table for spraying with BlueStar® the following are the results of the experiment after 30 days.

Painted Dry Wall, Cloth Material, White Paper, & Vinyl Floor Tile (Self Stick):

Luminol was applied to the substrate sample with positive results on Painted Dry Wall, Vinyl Floor Tile (Self Stick).

The Cloth Material reacted in the dilutions of 1:10, 1:100, 1:1,000, 1:5,000, & 1:10,000. However there was no reaction in the dilutions of 1:50,000, 1:100,000 and 1:1,000,000.

The White Paper reacted in the dilutions of 1:10, 1:100, 1:1,000, 1:5,000, 1:10,000, & 1:50,000. However there was no reaction in the dilutions of 1:100,000 and 1:1,000,000.

BlueStar® was applied to the substrate sample with positive results on all dilutions.
BlueStar® was far superior than Luminol on this sample, because of the brightness of the reaction and the reactivity itself being more reactive than the Luminol on 2 of the samples.

Wood (White Pine):

Luminol was applied to the substrate sample with positive results on all the dilutions except for 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000.

BlueStar® was applied to the substrate sample with positive results on the dilutions of 1:10, 1:100, 1:1,000, and 1:5,000. There were no results in the dilution 1:10,000, 1:50,000, 1:100,000 1:1,000,000. (Figure #7)

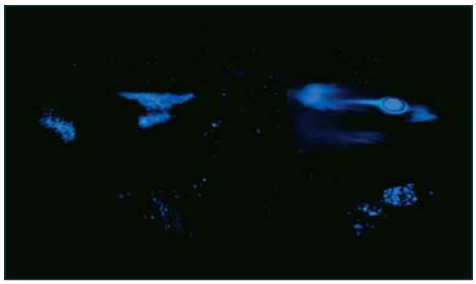


Figure #7

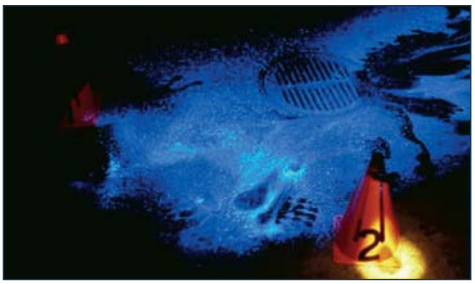


Figure #8

The BlueStar® and Luminol reacted at the same levels of dilution; however the BlueStar® in this sample was much brighter than the Luminol test.

Plywood 1/2 Inch:

Luminol was applied to the substrate sample with positive results on the dilutions of only 1:10. There were no results in the dilutions any of the other dilutions.

BlueStar® was applied to the substrate sample with positive results on the dilutions of 1:10, 1:100, 1:1,000, and 1:5,000, with no results in the dilutions of 1:10,000 & 1:50,000, 1:100,000, 1:1,000,000.

The BlueStar® was much more reactive with results in three higher dilutions than Luminol.

Carpet:

Luminol was applied to the substrate sample with positive results on the dilutions of 1:10, 1:100, 1:1,000 & 1:5,000.

BlueStar® was applied to the substrate sample with positive results on all dilutions.

The BlueStar® out performed the Luminol in this sample with positive results in the 1:10,000 & 1:50,000, 1:100,000 and 1:1,000,000 dilutions and proved more sensitive and was much brighter than the Luminol test.

Clean Up:

Testing was also conducted on possible blood stains with bleach as a cleaning agent and the effects of Luminol and BlueStar® Forensic. Both Luminol and BlueStar® Forensic reacted with the bleach with a very short hot reaction (Figure #8), which peaked and then dissipated almost immediately. No difference was noted between the Luminol and the BlueStar® Forensic with the reaction to bleach and not the regular reaction to the blood was detected.

Conclusion:

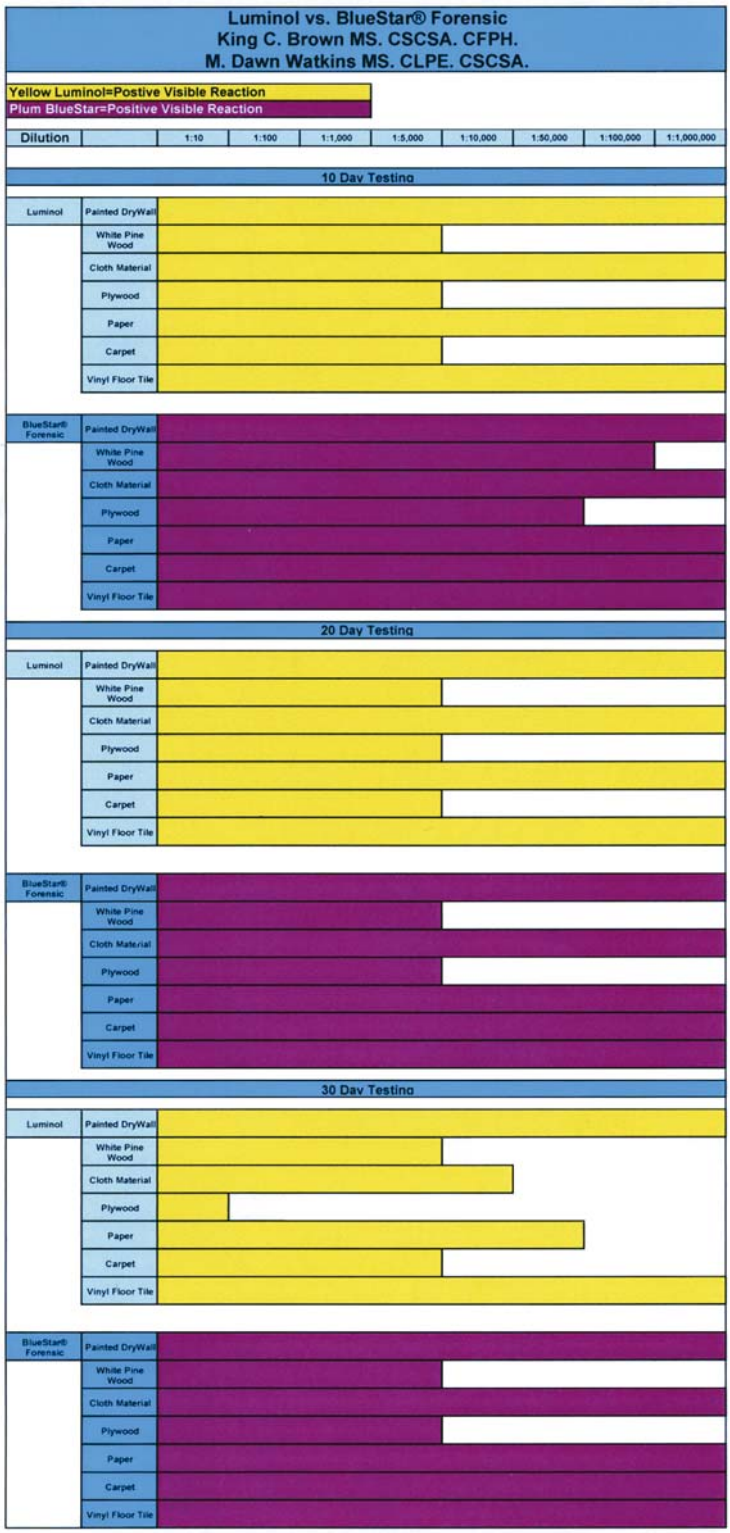
After testing the Luminol and BlueStar® Forensic on blood stains which were 10, 20 & 30 days old and placed on various substrates, it is very apparent that the BlueStar® Forensic outperformed the Luminol on all samples. This is evident by examining the Luminol vs. BlueStar® Forensic Chart (Figure #9). To further continue the testing on the carpet samples BlueStar® Forensic was sprayed on the same sample as the Luminol was sprayed on after 30 days, the BlueStar® Forensic reacted with the carpet sample in all dilutions. This displays the probability that BlueStar® Forensic can be used

Figure #10

after Luminol for the possibility of better results and as a secondary confirmatory test.

Photography of both Luminol and BlueStar® Forensic with a high resolution Digital Camera (Nikon D-100 used for this experiment) set at ISO 1600 was used to capture the reactions at approximately 15 to 30 seconds of exposure. The day 30 experiment was photographed with bounce lighting on the BlueStar® Forensic with excellent results (Figure #10).

Figure #9



After working with Luminol for several years and Luminol having a long history, a new reactive chemical for the detection of hidden blood is BlueStar® Forensic. The findings in these experiments have proven that BlueStar® Forensic is easier to use, more sensitive, superior to Luminol, easier to photograph.

Dispose of unused Bluestar® Forensic solution in a sink under running water.

A validation study (1) conducted by the Genetic Prints Functional Unit of the Nantes University Hospital (CHU-France) demonstrated the possibility to still perform DNA typing with blood dilutions of 1/1000 on dried blood, which is enough , since the most sophisticated DNA extraction methods (PCR amplification) do not yield any genetic profiles at blood dilutions over1/200. [5]

A validation study (2) conducted by the "Institut de Recherche Criminelle de la Gendarmerie Nationale" (IRCGN) of the French National DefenseDepartment. Upon extensive testing, they demonstrated that DNA typing is still reliably possible 30 days after the repeated treatment of bloodstains with Bluestar® Forensic "training" formulas does destroy DNA and is therefore intended only for training purposes. Do not use Bluestar® Forensic "training" for real investigations. [5]

Refer to the Material safety data sheets (MSDS) for the Bluestar® Forensic "training" tablets and working solution are available in PDF format under the Bluestar® Forensic web site :
www.bluestar-forensic.com/gb/download.php

Legend:

[1] BlueStar® Forensic Information Page :
http://www.crimescene.com/store/index.php?main_page=product_info&products_id=130
[2] BlueStar® Forensic Home Page :
http://www.bluestar-forensic.com/gb/index.php
[3] Samantha K. Webb. "Luminol vs. BlueStar® Forensic : A Comparison Study of Latent Blood Reagents"
[4] Ted Yeshion, MS. Detecting Blood at the Crime Scene 1991 Course Manual.
[5] BlueStar® Forensic Instruction Manual
1- Results of the Nantes CHU validation study can be downloaded on their web site : www.bluestar-forensic.com/gb/download.php
2- An I.R.C.G.N. presentation and their validation study report can be downloaded on their web site : www.bluestar-forensic.com/gb/download.php
[6] BlueStar® Forensic CD

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