Luminol vs. BlueStar®: A Comparison Study of Latent Blood Reagents

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Abstract

The luminol test is a presumptive test for blood that many crime labs employ in crime scene investigation. A new product, the BlueStar® Latent Blood Reagent, was tested and compared to luminol in a series of three different studies. The first study was performed to see how age and temperature variance would affect results for various substrates. The second study was done to better understand how bleach cleanup of stains would be visualized with both luminol and BlueStar® on various substrates. The third and final study involved the addition of glycine to both reagents to try to reduce the effects of bleach interference.

BlueStar® Background

Similar to luminol, BlueStar® utilizes hemoglobin's peroxidase-like activity. The manufacturer, Roc Imports, claims that BlueStar® is extremely sensitive and that it is long lasting. Also, it can be applied numerous times and still receive DNA typing results. Roc Imports also claims that BlueStar® is the most sensitive presumptive blood test on the market.

Luminol and BlueStar® General Application Procedures

Luminol **(Kits purchased through Doje's Incorporated)**

Reagent Preparation:

1. Add 8oz. distilled water to a mixing container.
2. Add one vial of Sodium Perborate to the water.
3. Cover the mixing container and shake for about 15 seconds.
4. Add one vial of Luminol and Sodium Carbonate.
5. Cover the mixing container and shake.
6. Allow the undissolved particles to settle to the bottom.
7. Pour solution into the sprayer.
8. Always mix chemicals just prior to use and do not use after 8 hours.
    a. Note that this time frame changed to 20 minutes in the second and third studies when more of the reagent was purchased and the manufacturer had changed the directions.

Procedure:

1. Set spray bottle to the finest spray possible.
2. If photographing, make sure all equipment (tri-pods, cameras, etc.) is set up.
3. Darken the room as much as possible and let your eyes adjust before spraying.
4. Make sure you are able to back away from where you are spraying.
5. Spray trigger while using a sweeping motion from side to side.
6. Immediately check for a bluish luminescence.
7. Photograph the luminescence if required.

Photography:

Make sure that a tripod is set up before spraying and the cameras are loaded and ready. 400 ASA film is recommended. Use a "glow-mark" ruler as a reference when photographing. If one is not available, attempt to re-photograph without moving the camera when there is appropriate lighting to use a regular ruler. Use a 35mm camera or digital camera with f 1.4 or faster, set wide open. Set shutter to "bulb setting". Exposure time should be approximately 30 seconds.

BlueStar®

Reagent Preparation:

1. Add 125 mL (4oz.) of distilled water to a spray bottle.
2. Add a pair of BlueStar® tablets to the water. If you need more, use 125 mL distilled water per pair of tablets.
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.

Procedure:

1. Set spray bottle to the finest spray possible.
2. If photographing, make sure all equipment (tri-pods, cameras, etc.) is set up.
3. Darken the room as much as possible and let your eyes adjust before spraying.
4. Make sure you are able to back away from where you are spraying.
5. Spray trigger while using a sweeping motion from side to side.
6. Immediately check for a bluish luminescence.
7. Photograph the luminescence if required.

Photography:

See luminol procedure.

Luminol vs. BlueStar® Initial Study

N. L. Gavrilovic, B.A. and S. K. Webb, B.S.

Procedure

Serial dilutions of blood were made from purple top blood tubes received from the City of Saint Louis Office of the Medical Examiner. Distilled water was added to create dilutions of 1:10, 1:100, 1:1,000, 1:10,000, 1:100, 000 and 1:1,000,000. Stains were made on the various substrates on days so that a four day study, a four week study and a seven week study could be accomplished. A sponge was used to apply the blood dilutions. The substrates were carpet, white linoleum, tan linoleum, floor board, dry wall, red bricks, pre-treated oak floor, black and white tiles and wood. One set of substrates were kept in our lab. These were known as our cooled substrates. The other group was placed in a non-air-conditioned part of our building and was known as the heated environment. The study was done over the summer when temperatures in the "heated environment" were typically in the nineties. A total of three sprayings were completed. The substrates were divided so that one side was sprayed with luminol and the other was sprayed with BlueStar® consecutively. Photographs were taken of the substrate before the reaction and after spraying while in the dark.

Conclusion

In the four day study, stains diluted to 1:100,000 and 1:1,000,000 were generally not seen with the exception of the dry wall and the black and white tiles. Luminol appeared to have better results with hotter temperatures rather than the colder. The BlueStar® showed little to no difference with the temperature change.

In the four week study, dilutions of 1:10,000, 1:100,000 and 1:1,000,000 did not give a positive reaction except with drywall and the black and white tiles. The temperature difference and age produced variable results.

For the seven week study, dilutions of 1:10,000, 1:100,000 and 1:1,000,000 were not seen except with the carpet and the pre-treated flooring. The age of the stains had no impact on results.

In the four week and seven week studies we found that the cooler temperature produced better results. There was no major difference in reaction when comparing older and newer stains. Also, the substrates showed variable results. BlueStar® reacted as well as or better than luminol on all substrates in all three studies. Overall, BlueStar® outperformed luminol because of its ability to detect more dilute stains.

Luminol vs. BlueStar® Bleach Cleanup Study

N. M. Ford, B.A. and S. K. Webb, B.S

Procedure

Serial dilutions of blood were made in distilled water from purple top blood tubes received from the City of Saint Louis Office of the Medical Examiner. The dilutions were 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000. Stains were made on various substrates by placing two drops of blood from a dropper. Pure Clorox bleach was used to clean the appropriate substrates on the day of the spraying and substrates were cleaned until there was no visible trace of blood. Stains were made so that a 1 week, 2 week, 4 week, 6 week, and 8 week study could be accomplished. The substrates used were carpet, blue linoleum tile, black linoleum tile, gray ceramic tile, red brick patio stones, dry wall, oak floorboard, pine plywood, pine 2x4 board, treated oak floor, and white wall paneling. There were four separate groupings with this experiment: blood tested with luminol, blood cleaned with bleach and tested with luminol, blood tested with BlueStar® and blood cleaned with bleach and tested with BlueStar®. One spraying was completed and each substrate was sprayed with either the Luminol or the BlueStar® reagent. Photographs were taken of each test before spraying and again after spraying while in the dark.

Conclusion

The purpose of this experiment was to determine the effect of bleach on the ability of luminol and BlueStar® to detect blood. There were several general trends that were exhibited by all the tests. The first thing to note is that blood was not detected on any of the substrates in the 1:100,000 and 1:1,000,000 dilutions. In almost every case the luminescence of the BlueStar® lasted longer than the luminescence of the luminol. When bleach was used there was a general sparkling that appears in a wipe pattern for both reagents. Finally, all the substrates that were treated with bleach did not show as strong results as those not treated.

In five of the ten substrates, positive results were obtained when bloodstains were treated with bleach before they were tested with BlueStar®. Only three of the substrates tested with luminol showed positive results after being treated with bleach. In all these cases, however, the results obtained only occurred with the 1:10 and 1:100 blood dilutions.

Bleach clearly has a destructive effect on blood that makes it much more difficult to detect minute traces of blood. However, the BlueStar® reagent had better success with detecting blood treated with bleach. The luminescence of BlueStar® was longer lasting and brighter than luminol, and therefore made it easier to find blood stains despite interference from bleach. BlueStar® is the better choice when trying

to detect blood when bleach is involved. However, it is only useful in cases where there is a higher concentration of blood present.

Luminol vs. BlueStar®: Addition of Glycine to Reduce the Interference of Bleach

N.M. Ford, B.A. and S. K. Webb, B.S

Procedure

First, a serial dilution of a sample of blood was made so that there were six concentrations including 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000 and 1:1,000,000. The staining took place over a six week period, and during this time the substrates were stained four times. The first stains made corresponded to stains that were six weeks old. The next stains were four weeks old, followed by the final two stains that correspond to two and one week old stains. Stains were made by placing two drops of each blood dilution on each substrate. The substrates that were used were drywall, plywood, sub-flooring, 2x4 boards, floor board, carpet, paneling, and blue linoleum tile. There were a total of four tests performed on each substrate. The first two were blood tested with luminol and glycine mix and BlueStar® and glycine mix. These served as controls. The next set of substrates were bloodstained and then cleaned with bleach. Bleaching occurred on the day of the spraying and substrates were cleaned until there were no visible traces of blood. They were then sprayed with luminol and glycine mix. The last set of substrates had blood cleaned with bleach and they were then sprayed with BlueStar® and glycine mix.

The luminol and glycine solution was prepared as follows. After preparation of the luminol reagent, 0.469g of glycine was added and mixed. The pH of the entire solution was then brought to 12, and the undissolved particles were allowed to settle before the solution was poured into a sprayer.

The BlueStar® reagent was prepared as follows. Two BlueStar® tablets were added and allowed to dissolve for two minutes by stirring gently. Once the tablets were dissolved, 0.469g of glycine was added and mixed. The pH of the solution was then brought up to 12 and poured into a spray bottle.

After both sprays were ready, each substrate was sprayed in the dark with its corresponding reagent. Then a photograph of the results was taken.

Conclusion

The purpose of this experiment was to determine if the chemiluminescent interference caused by bleach could be reduced by the addition of glycine to the BlueStar® and luminol reagents. Luminol and BlueStar® controls were both effective in producing positive results in all but one of the substrates for the 1:10, 1:100, and 1:1,000 blood dilutions. Therefore we can conclude that the addition of glycine does not inhibit the detection of blood. As for glycine's effect on reducing interference by bleach there was a

noticeable difference. On the substrates treated with bleach there was an initial bright wipe pattern, but after a few seconds it fades into a dull blue background. This allowed for better detection of blood stains that would normally be difficult to distinguish. In this experiment three of the eight substrates had positive results for the 1:10 blood dilution after being cleaned with bleach. One positive result was seen for the 1:100 dilution after being cleaned with bleach and sprayed with BlueStar®. These three substrates had positive results with luminol and with BlueStar®. The addition of glycine definitely reduces the chemiluminescent interference of bleach, which facilitates blood detection. However, the preparation of the reagent, including the pH meter usage, may cause investigators to utilize this procedure only under special circumstances.

References

Cluefinder, Inc., Cluefinders Master Blood Detection Kit, Instruction Sheet, 1986.

Gross, Harris and Kaldun. "The Effect of Luminol on Presumptive Tests and DNA Analysis Using the Polymerase Chain Reaction", Journal of Forensic Sciences, JFSCA, Vol. 44, No. 4, July 1999, pp 837-840.

James, Stuart. Interpretation of Bloodstain Evidence at Crime Scenes, CRC Press, 1999.

Kent, Erina, Douglas A. Elliot and Gordon Miskelly. "Inhibition of Bleach-Induced Luminol Chemiluminescence." Journal of Forensic Sciences, Vol. 48, No. 1, January 2003: 64-67.

Lytle, L.T., and D.G. Hedgecock. "Chemiluminescence in the Visualization of Forensic Bloodstains", Journal of Forensic Sciences, JFSCA, Vol. 23, No. 3, July 1978, pp 550-562.

Roc Import, BlueStar® Latent Blood Reagent, Instruction Booklet, 2003.

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