Technical Paper

Katherine Martin, Ada County Sheriff's
Office Intern

The Effects of Bluestar on Presumptive Tests for Blood

Abstract:

Presumptive tests for blood need to be sensitive in order to detect blood that is not readily visible. Furthermore, presumptive tests for blood have different applications and sensitivities. One presumptive test that is particularly useful to a crime scene responder is Bluestar. Bluestar is a reagent that uses chemiluminescence and is aerosolized through a spray bottle covering large areas. Bluestar is practical for crime scene use because it can detect blood in large areas, but has drawbacks with shelf life and false positives. Thus, other presumptive tests for blood have a role in crime scene investigation. This study looked at the sensitivities of Bluestar and three other presumptive tests: leucomalachite green, phenolphthalein, and ortho-tolidine. The study also looked at how applying Bluestar first may affect the results of the other tests. The sensitivities were studied with serial blood dilutions ranging from 1:1 to 1:1,000,000. Bluestar was found to be the most sensitive detecting blood at 1:100,000 dilution. Furthermore, Bluestar decreased the sensitivities of the leucomalachite green and ortho-tolidine test, when Bluestar was added to the blood before the other reagents.

Introduction

In order to distinguish Bluestar from the other tests in this study, Bluestar will be called a preliminary test. Bluestar is a preliminary test because it can detect blood that is at very low levels, but Bluestar is not specific to blood. Bluestar can react with other chemicals such as bleach and result in a false positive. Thus after Bluestar is used, an additional presumptive test is performed to specify the sample as blood. Bluestar and most presumptive tests for blood use the peroxidase-like activity of hemoglobin in blood to cause a visible color change. Sensitivity is the boundary of when there is still enough heme to react with the reagent even though the solutions are dilute. Since presumptive tests can detect blood that could be obscured by cleaning and other means, they are very useful in forensic analysis.

This study was designed to test the sensitivity of Bluestar and three presumptive tests for blood: leucomalachite green, ortho-tolidine, and phenolphthalein. It also addressed whether applying Bluestar first interfered with the remaining three tests when they were subsequently applied. A range of 1:1 to 1:1,000,000 was chosen for the serial dilutions since previous studies had shown the sensitivities to land in this range. It was also shown that the nature of the surface the blood was on could have an effect on the sensitivity of the test [1]. Thus to eliminate the surface as a factor, this study looked at liquid blood dilutions in test tubes.

Bluestar is often applied first because it is a spray that can cover large areas at one time. In the presence of blood, a blue chemiluminescence (near 430nm) is observed. The blue light of the reaction is emitted when the reagent reacts with the iron in the hemoglobin [2]. Since Bluestar is a spray and the other three tests are applied as drops, the volume of reagent sprayed in a typical application was converted to volume in liquid drops that could be added to a test tube. This was done by measuring the liquid volume which came out of spray bottles in a typical application. The purpose of this study was twofold:

first to evaluate the sensitivity of Bluestar to liquid dilutions of blood, and secondly to identify if the application of Bluestar had any adverse effects on subsequent presumptive blood reagents.

Materials and Methods

The initial part of the study was to determine how much blood and reagent were to be used. The number of drops of Bluestar was determined through a study of different spray bottles. Two different spray bottles were filled with water. Per the manufacturer's recommendation for the area to be sprayed, each was sprayed five times directly over a test tube to replicate the recommendation. The water was then pulled up in a pipette, and dropped out while the drops were counted.

This procedure was repeated four times per bottle. The average volume was equal to eight drops of water per five sprays.
Thus eight drops of Bluestar was chosen to replicate the typical Bluestar application which was added in each sample test tube.

Serial dilutions of cow blood were made using an Eppendorf pipette that was rated for 100µL to 1000µL. The dilutions were 1:1, 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000. This range was chosen based on previous studies with Bluestar [2]. 1 mL sample aliquots were chosen based on the Eppendorf pipette minimum of 100µL. The blood dilutions sat capped in test tubes at room temperature for two weeks to homogenize and age.

After the two weeks, Bluestar was prepared according to manufacturer's specifications. After it was made, it reacted positive on a known sample. To confirm the manufacturer's instructions that Bluestar needs to be made fresh, two week old Bluestar was tested on a known sample and no reaction was seen. Pictures were taken of the test tubes in a row to see the dilution. Pictures were also taken of each test tube before the Bluestar was added. There were two sets of serial blood dilutions. One set would have Bluestar added, and the other would not. A positive control was a known blood sample, and a negative control of water was used. The procedure was to add the eight drops of Bluestar, take a picture, and then swab the test tube. The swab was submerged and swirled around. It was noted whether or not the Bluestar reaction was observed. The test tubes that did not get Bluestar were also swabbed. The swabs were then allowed to dry in a drying chamber without contamination, and the test tubes were capped.

After three days, when the swabs were fully dry, they were each cut into three pieces. Each piece was then tested with a different blood reagent: phenolphthalein, ortho-tolidine, and leucomalachite green. The phenolphthalein was in a pre-manufactured tube that was first broke and mixed, and it then reacted positive with the positive control. The leucomalachite green and ortho-tolidine were previously made in the lab. The leucomalachite green reacted positively with the control without the oxidant of hydrogen peroxide. The ortho-tolidine reacted positive with the hydrogen peroxide. Each piece of the swabs received a drop of one of the reagents. The leucomalachite green and ortho-tolidine were each followed by a drop of hydrogen peroxide, and it was noted if the reaction was seen without the hydrogen peroxide.

The test tubes with and without the Bluestar sat capped for two weeks. They were then swabbed again, and the same process of testing the swabs was carried out. All results were recorded.

Results

The Bluestar reaction was observed up to the 1:100,000 dilution in the immediately prepped blood samples. Leucomalachite green detected the blood at 1:100 with the Bluestar application and 1:1,000 without the Bluestar in the blood. Phenolphthalein reacted at 1:100 with and without the Bluestar application. Ortho-tolidine detected blood at 1:10,000 with and without Bluestar. After the two week waiting period, the leucomalachite green tested at the same levels as when applied to the immediately prepped samples. However, the ortho-tolidine levels changed to 1:100 with Bluestar and 1:1,000 without

Bluestar application. Phenolphthalein also tested lower with the Bluestar at 1:10 compared to 1:100 without. See tables in the appendix for full results.

Discussion

Bluestar was the most sensitive test as chemiluminescence was observed with the 1:100,000 dilution. Though Bluestar was the most sensitive, it is not the most practical in all cases. Since Bluestar must be used within a few hours of preparation, it would not be practical for small areas because a lot of the solution would be wasted. For small stains or areas, ortho-tolidine would be more practical because it has a longer shelf life than Bluestar, and it has a higher sensitivity than the leucomalachite green and the phenolphthalein. Though it should be noted that ortho-tolidine has some health concerns as a possible carcinogen. Bluestar affected the ability of the leucomalachite to detect blood initially, but did not affect ortho-tolidine and phenolphthalein's ability to detect blood in the first phase of the study. The effect of Bluestar was seen immediately with the leucomalachite green, since the reagent could detect one dilution further without the Bluestar application. The effect of Bluestar on the ortho-tolidine was seen after the two week waiting period. The ortho-tolidine dropped two levels with the Bluestar and one level in the blood without the Bluestar. This suggests that ortho-tolidine itself is not as sensitive with older blood. Phenolphthalein was the least sensitive across the board, and dropped a level of sensitivity with Bluestar in the blood after two weeks. Leucomalachite green fell in the middle range of sensitivity, and the sensitivities did not change with time. All presumptive tests for blood have their place, and the context of each crime scene will help determine which presumptive test should be used as coverage area and age of the blood will play a role.

Conclusion

This experiment assessed the sensitivity of Bluestar and how it affected the other presumptive tests that could be used after the Bluestar was applied at a crime scene. Eight drops of Bluestar were added to the test tubes because testing showed eight was the average. Bluestar also needs to be made fresh. Bluestar was more sensitive than the three other presumptive reagents tested. Furthermore, Bluestar affected the sensitivity of the leucomalachite green at the beginning. It also affected the sensitivity of the ortho-tolidine and phenolphthalein after the Bluestar sat for two weeks. Future experiments could test where in between 1:100,000 and 1:1,000,000 does Bluestar no longer detect the blood. Studies could also look at allowing the Bluestar to sit in the blood for a longer period of time. Overall, the initial application of Bluestar only caused minimal effects on the other presumptive tests.

References

1. Luedeke, M.; Miller, E.; Sprague, J. technical Note: The effects of Bluestar and luminol when used in conjunction with tetramethylbenzidine or phenolphthalein. Forensic Science International. 2016, 262, 156-159.

2. Vaughan, J. The Effects of Bluestar on the Kastle-Meyer Presumptive Test for Blood. Journal of Forensic Identification. 2011, 61(1), 38-49.

Appendix

Table 1: Bluestar Reaction

Dilution	1:1	1:10	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	Water
Bluestar Reaction Seen	Yes	Yes	Yes	Yes	Yes	Yes	No	No

Table 2: Initial Presumptive Tests After Bluestar Applied

Dilution	LMG Reaction	PH Reaction	OT Reaction
Known	Yes w/o H2O2	Yes	Yes
1:1 w/ BS	Yes w/o H2O2	Yes	Yes
1:10 w/ BS	Yes w/o H2O2	Yes	Yes
1:100 w/ BS	Yes w/o H2O2	Yes	Yes
1:1,000 w/ BS	No	No	Yes
1:10,000 w/ BS	No	No	Yes
1:100,000 w/ BS	No	No	No
1:1,000,000 w/ BS	No	No	No
1:1,000,000 w/o BS	No	No	No
1:100,000 w/o BS	No	No	No
1:10,000 w/o BS	No	No	Yes
1:1,000 w/o BS	Yes	No	Yes
1:100 w/o BS	Yes w/o H2O2	Yes	Yes

1:10 w/o BS	Yes w/o H2O2	Yes	Yes
1:1 w/o BS	Yes w/o H2O2	Yes	Yes
water	No	No	No

Key:
BS= Bluestar
LMG= Leucomalachite Green
PH= Phenolphthalein
OT= ortho-tolidine
H2O2= Hydrogen Peroxide
w/= with
w/o= without

Table 3: Presumptive Tests after Two Weeks with Bluestar

Dilution	LMG Reaction	PH Reaction	OT Reaction
Known	Yes w/o H2O2	Yes	Yes
1:1 w/ BS	Yes w/o H2O2	Yes	Yes
1:10 w/ BS	Yes w/o H2O2	Yes	Yes
1:100 w/ BS	Yes	No	Yes
1:1,000 w/ BS	No	No	No
1:10,000 w/ BS	No	No	No
1:100,000 w/ BS	No	No	No
1:1,000,000 w/ BS	No	No	No
1:1,000,000 w/o BS	No	No	No

1:100,000 w/o BS	No	No	No
1:10,000 w/o BS	No	No	No
1:1,000 w/o BS	Yes	No	Yes
1:100 w/o BS	Yes w/o H2O2	Yes	Yes
1:10 w/o BS	Yes w/o H2O2	Yes	Yes
1:1 w/o BS	Yes w/o H2O2	Yes	Yes
water	No	No	No

Key:
BS= Bluestar
LMG= Leucomalachite Green
PH= Phenolphthalein
OT= ortho-tolidine
H2O2= Hydrogen Peroxide
w/= with
w/o= without