JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, 2018
doi: 10.1111/1556-4029.13914
Available online at: onlinelibrary.wiley.com

TECHNICAL NOTE

CRIMINALISTICS

Joshua L. Adams,[1,2] *Ph.D.; Emily D. Rancourt*,[2] *M.S.; and Angi M. Christensen*,[2,3] *Ph.D.*

The Effect of Household Oxidizing Cleaners on Chemiluminescence of Blood Using Bluestar®†

ABSTRACT: This study tests the effect of three common oxidizing cleaners on the ability of the Bluestar Forensic® presumptive test for blood to identify the presence of blood on ceramic tile after cleaning. The cleaners tested were Lysol®, OxiClean®, and Arm & Hammer®. This study also tested which cleaner was the most effective at removing blood, measured by the intensity of chemiluminescence, which was quantified using RGB values in ImageJ. A "hasty" 1-min cleaning of a blood droplet was simulated using the three cleaners. The chemiluminescence of the Bluestar® reactions after cleaning the blood-treated region was compared to an untreated region of the same tile for each cleaner, as well as to the treated regions of tiles between the three cleaners. Results indicate that none of the three cleaners removed all of the blood (all $p < 0.001$) and that Lysol® removed more blood compared to the OxiClean® and Arm & Hammer®.

KEYWORDS: forensic science, crime scene investigation, Bluestar®, blood, oxidizing cleaners, crime scene alteration

Perpetrators of a crime sometimes attempt to remove or destroy evidence that may link them to their crime. It is therefore important for crime scene investigators (CSIs) to understand the effects of certain scene-altering activities on the evidence they are attempting to locate. For example, suspects may try to clean or remove blood that may link themselves or their victims to the crime scene. Remnants of blood found (or not found) at a scene can significantly affect lead development at a crime scene, such as the ability to link a suspect or a victim to the crime scene. CSIs frequently use presumptive field tests to detect the presence of blood. One popular option is Bluestar Forensic®, which produces a chemiluminescent reaction with blood, allowing investigators to visually identify the presence of blood. This study examined whether Bluestar® can identify the presence of blood on ceramic floor tiles following the use of three cleaning products.

There have been numerous studies on false-positive/false-negative rates for detecting blood using chemical presumptive tests (1–9). One study utilized active oxygen cleaning products to examine several presumptive blood test products including luminol, phenolphthalein, and HexagoN OBTI® on laundered fabrics (1). Results indicated that washing fabrics (cotton, jeans, and toweling) with products containing active oxygen could prevent presumptive and human hemoglobin tests from giving a positive reaction, regardless of the temperature of the water used. Another study involved laundering several fabrics that were stained with blood and then washed with 98% sodium percarbonate (2). Results indicated that Bluestar® was still able to detect the presence of blood from a laundered cloth at dilutions lower than $1{:}10^2$. Both studies advocated the need to further study household cleaning products, since they are continually changing. Bluestar® has been shown to be better at detecting diluted concentrations of blood compared to luminol (3). One recent study examined the use of several household cleaners in an attempted crime scene alteration scenario involving bloodstains on carpet, ceramic tile, and press-on vinyl tile (10). Results indicated that Lysol Power & Free Multi-Purpose Cleaner with Hydrogen Peroxide® produced a false-negative reaction with Bluestar® on all substrates. It is apparent that additional research on the effects of cleaning products on presumptive blood tests is needed.

Materials and Methods

Twenty-four (24) 1′x1′ Daltile® brand ceramic floor tiles (color BT01) were used in this study. Ceramic tile was selected because it is commonly found in bathroom or kitchen areas where crime scene alteration may be attempted. The three cleaners tested were Lysol® with Hydrogen Peroxide Multi-Purpose Cleaner, OxiClean® Versatile Stain Remover, and Arm & Hammer® Super Washing Soda Detergent Booster. To ensure that the tiles did not have inherent chemiluminescent properties or

[1]United States Army Criminal Investigation Command, 105 Fenton Circle, Bldg 305, Joint Base Myer-Henderson Hall, VA, 22211.
[2]Department of Forensic Science, George Mason University, 4400 University Drive, Fairfax, VA, 22030.
[3]Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, Quantico, VA, 22135.
Corresponding author: Joshua L. Adams, Ph.D. E-mail: adams.joshua1@gmail.com

†Names of commercial manufacturers are provided for identification purposes only, and inclusion does not imply endorsement of the manufacturer or its products or services by the United States Army Criminal Investigation Command (USACIDC) or the Federal Bureau of Investigation (FBI). The views expressed are those of the authors and do not necessarily reflect the official policy or position of the USACIDC or FBI.

Received 30 June 2018; and in revised form 3 Aug. 2018; accepted 30 Aug. 2018.

chemiluminescent surface contaminants that might affect test results (negative control), a sample tile was first treated with Bluestar® and examined for any chemiluminescence; none was noted qualitatively. Each cleaner used was treated with Bluestar® to ensure they did not have inherent chemiluminescent properties; none were noted. In addition, a tile with a bloodstain with no cleaner was treated with Bluestar®; a positive chemiluminescent reaction was observed (positive control).

One blood droplet (approximately 50 μL) was applied to each tile from a height of 5 feet using a plastic pipette and a step ladder, and allowed to dry for 1 h. The blood had been previously refrigerated at 3°C, and the room temperature was between 4 and 7°C during the study. The size of the bloodstain made from the blood droplet on the target surface was roughly circular and approximately 15 mm in diameter. After the blood was dry, a 79 × 79 mm circle was drawn around the blood droplet using a permanent marker and a compass drawing tool. Eight tiles were then each cleaned using one of the three oxidizing cleaners.

The OxiClean® was prepared by mixing 1 full manufacturer provided scoop with 1 gallon of warm water per manufacturer's instructions. The Arm & Hammer® was prepared by mixing ½ cup of Arm & Hammer® powder in 1 gallon of warm water per manufacturer's instructions. The Lysol® did not require any preparation because it came premixed in a manufacturer's atomizer spray bottle. Cleaning was performed using a disposable toothbrush (one used for each tile to prevent cross-contamination) with one of the three cleaning agents. To apply the OxiClean® and Arm & Hammer®, the toothbrush was dipped into the prepared solution. The Lysol® was applied by squeezing the spray bottle atomizer three times. Each tile was then cleaned with a toothbrush in a circular motion for one minute with approximately the same amount of force used to brush one's teeth. Each tile was then wiped dry with an individual paper towel. This cleaning method was selected to simulate a "hasty" cleaning, such as might be used by the perpetrator of a crime attempting to quickly remove evidence.

Following cleaning, no blood was visually apparent on any of the 24 tiles. The Bluestar® reagent was then applied to the tile surface using three full sprays from the manufacturer-supplied bottles. Each tile was then photographed. All photographs were taken with a Nikon D5600 DSLR, 55 mm lens, with a tripod and remote. Each photograph was exposed for 10 sec, at an *f-stop* of 5.6, and ISO of 400. The camera-to-sample distance was kept constant at 2′ 2″. The photographs were taken in a partially darkened setting, consisting of a household garage with the exterior garage doors closed, interior lights off, and partly cloudy daytime conditions outside.

Images were then uploaded into ImageJ (11), (a public domain, Java-based image processing program developed at the National Institutes of Health) for quantification of chemiluminescence. Chemiluminescence was measured following a method described by previous researchers who quantified fluorescence of bone samples using RGB values (12,13). A standardized circular region of interest (ROI) of 1500 × 1500 pixels (approximately 70 mm) was selected from four different locations in each image. The size of the ROI was selected because it is slightly smaller than the size of the circle drawn around the blood droplet, allowing the ROI to fit within the circle while not including any of the black ink. One of the ROIs was within the circle that circumscribed the location where the blood droplet was applied and then cleaned, and the other ROIs were selected from three outlying regions of the same tile approximately 150 mm from the circle in the image, representing an area that was not treated with blood (Fig. 1). The brightness of the ROI was determined using the Analyze/Measure tool which converts RGB pixel values into brightness values using the (unweighted) formula $V = (R+B+G)/3$. This value represents the intensity of chemiluminescence of the selected region. Paired *t*-tests were used to compare the mean RGB values of the untreated and the treated/cleaned regions for each tile. The differences in RBG values of the treated/cleaned regions between the three products were also assessed.

Results

The variation in RGB values for the three untreated regions was very small, so these values were averaged for subsequent analyses (Lysol's® standard error of the mean for the outer areas of the eight tiles ranged from 0.79 to 2.90 RGB, OxiClean® ranged from 0.45 to 3.72, and Arm & Hammer® ranged from 0.44 to 2.75). Cleaning with Lysol® resulted in lower levels of chemiluminescence with Bluestar® as compared to Arm & Hammer® and OxiClean®, which both still displayed significantly visible chemiluminescence (Fig. 2). Summary statistics for RGB values for each cleaner and paired t-test results for untreated and treated/cleaned areas are shown in Table 1. For each cleaner, the RGB values were significantly greater for the regions that were treated with the blood droplet and then cleaned as compared to regions that were not treated with blood. This indicates that all three cleaners failed to completely remove all of the blood from the tile.

The RGB values for the treated/cleaned regions were also compared between the three cleaning products. Because the RGB values were not consistent between the three cleaners for the untreated regions (perhaps due to photography conditions or effects of background chemiluminescence), the RGB values of the treated/cleaned regions could not be directly compared and were instead evaluated as a percent change in RGB value between untreated and treated/cleaned regions. Lysol® had the smallest change in RGB value between the untreated region and the treated/cleaned region, increasing in RGB by 6.2, or 6.7%. Arm & Hammer® and OxiClean® had roughly equivalent changes in RGB value, with Arm & Hammer® increasing in

FIG. 1—*Selection of ROI in ImageJ, represented by the dotted line; three such ROIs were measured for each tile; the solid line represents the original location of the blood droplet.*

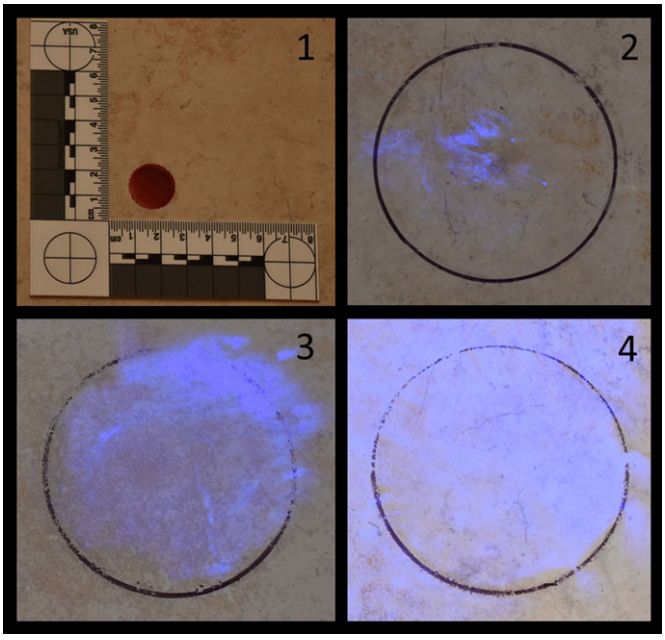

FIG. 2—*Examples of untreated blood droplet (1), and Bluestar® reaction to treated/cleaned regions after cleaning blood droplet with Lysol® (2), Arm & Hammer® (3), and OxiClean® (4).*

TABLE 1—*Comparison of RBG values for untreated and treated/cleaned regions of tiles using each cleaning product.*

Cleaner	Lysol®	Arm & Hammer®	OxiClean®
N	8	8	8
RGB value of untreated region (mean and SD)	91.95 (6.17)	119.0 (23.1)	146.4 (21.8)
RGB value of treated/cleaned region (mean and SD)	98.18 (7.14)	144.09 (21.24)	174.69 (22.38)
p (untreated versus treated/cleaned)	<0.001	<0.001	<0.001

RGB by 25.1 (20.7%), and OxiClean® increasing by 28.3 RGB (19.2%).

Discussion and Conclusion

In this study, all three cleaners failed to remove all of the blood, with the cleaned regions of the tiles quantitatively displaying greater chemiluminescence than regions that were never treated with blood. Of the three cleaners used in this study, Lysol® showed the smallest increase in RGB value between the untreated region of each tile and the treated/cleaned region of tile, indicating that it was the most effective of the three cleaners at diminishing the chemiluminescence produced by Bluestar®. The Arm & Hammer® and OxiClean® showed similar results, both not diminishing the chemiluminescence less than the Lysol®. Although studies have shown that sodium percarbonate is effective at diminishing the chemiluminescence of Bluestar® on laundered clothing (1), the sodium percarbonate found in Arm & Hammer® and OxiClean® was possibly not concentrated enough to diminish a chemiluminescent reaction.

Additional studies that use other types of substrates such as carpet, hardwood, and vinyl tile may be beneficial, as this study examined only one substrate, tile. Assessment of different and more thorough/deliberate cleaning techniques may also be useful, as would possibly increasing the drying time of the blood. In addition, it may be interesting to see the effects of Bluestar Forensic Magnum®, which has been shown to be more sensitive at detecting blood than Bluestar Forensic® (14), and could produce different results. It should also be noted that the results of this experiment are valid for only this type of substrate and the chosen environmental conditions.

In retrospect, the consistency of the data collected in this study may have been improved by using a completely darkened area to expose digital photographs of Bluestar® reactions. In this study, some ambient light was present, which may have introduced variance between photographs. Even though Bluestar® does not need complete darkness to be visible, complete darkness may have resulted in more consistent RGB values of the untreated regions in the photographs, possibly allowing a direct comparison of the treated/cleaned regions of different tiles. Background chemiluminescence could also have been a factor that affected RGB values.

These results indicate that none of the three products tested were able to completely remove blood from a tile; all three resulted in a chemiluminescent reaction with Bluestar® presumptive test for blood after cleaning. However, the Lysol® product significantly diminished chemiluminescence as compared to the OxiClean® and Arm & Hammer® products. If these cleaning products are found at a crime scene, CSIs should therefore be able to better interpret chemiluminescence findings. Specifically, if the Lysol® product is found and was potentially used to clean/alter the crime scene, CSIs should be aware that very little chemiluminescence may be produced when using Bluestar®.

Acknowledgments

We are grateful to the U.S. Army CID for providing the Bluestar Forensic® used in this project. This research received approval by the George Mason University Institutional Biosafety Committee (IBC) under Protocol Number 17-45.

References

1. Castello A, Frances F, Corella D, Verdu F. Active oxygen doctors the evidence. Naturwissenschaften 2009;96(2):303–7.
2. Castello A, Frances F, Verdu F. Chemistry in crime scene investigation: sodium percarbonate effects on bloodstains detection. J Forensic Sci 2012;57(2):500–2.
3. Dilbeck L. Use of Bluestar forensic in lieu of luminol at crime scenes. J Forensic Identif 2006;56(5):706–20.
4. Cox M. A study of the sensitive nature and specificity of four presumptive tests for blood. J Forensic Sci 1991;36(5):1503–11.
5. Creamer JI, Quickenden TI, Apanah MV, Kerr KA, Robertson P. A comprehensive experimental study of industrial, domestic, and environmental interferences with the forensic luminol test for blood. Luminescence 2003;18(4):193–8.
6. Creamer JI, Quickenden TI, Crichton LB, Roberson P, Ruhayel RA. Attempted cleaning of bloodstains and its effect on the forensic luminol test. Luminescence 2005;20(6):411–3.
7. Quickenden TI, Creamer JI. A study of common interferences with the forensic luminol test for blood. Luminescence 2001;16(4):295–8.
8. Seashols SJ, Cross HD, Shrader DL, Rief A. A comparison of chemical enhancements for the detection of latent blood. J Forensic Sci 2013;58 (1):130–3.

9. Pettolina M, Rainey J, Sanchez R. Using bluestar forensic to detect latent bloodstains under coats of paint. J Forensic Identif 2017;67(3): 341–53.
10. DiCarlo A, Jasra S. A qualitative evaluation of the effect cleaning products have on the Bluestar test for latent blood. J Emerg Forensic Sci Res 2016;1(1):49–59.
11. Rasband WS. ImageJ. Bethesda, MD: National Institutes of Health, 1997; https://imagej.nih.gov/ij/(accessed July 31, 2018).
12. Miranda GE, Haltenhoff MF, Franscisquini L, Daruge E. Use of an alternate light source to detect tooth and bone. Braz Dent J 2017;28(1): 78–81.
13. Swaraldahab MH, Christensen AM. The effect of time on bone fluorescence: implications for using alternative light sources to search for skeletal remains. J Foresnic Sci 2016;61(2):442–4.
14. Patel G, Hopwood A. An evaluation of luminol formulations and their effect on DNA profiling. Int J Legal Med 2013;127(4):723–9.