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Abstract

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Keywords

forensic science, luminol, chemiluminescence, blood, bleach, paint

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Introduction

The presence of blood at a crime scene can provide investigators with a treasure trove of information regarding the nature and circumstances of a particular crime and aid in crime scene reconstruction. Blood, like many other forms of forensic evidence, is a silent yet powerful witness that often reveals a plethora of valuable details otherwise unknown, such as the precise location where a crime initially occurred, the physical orientation of individuals when blood was deposited, the specific type of weapon or object used in the commission of a crime, the number of blows inflicted upon an individual by a blunt force object, as well as the individualization of the suspect or victim via nuclear DNA (nDNA) located in leukocytes (white blood cells). While there are scenarios where the presence of blood can be logically assumed without the need for presumptive and confirmatory testing (e.g., a deceased victim with multiple stab wounds and covered in reddish-brown stains), there are also many scenarios where reddish-brown stains cannot be confidently assumed to be blood. Consequently, the development of color-based presumptive tests, such as the Kastle-Meyer or Hemastix[®] test, along with crystal-based confirmatory tests, such as the Teichmann and Takayama test, make it possible for crime scene investigators and forensic scientists to quickly and easily identify ambiguous stains. One facet shared between these four tests is that they are generally employed once a stain has been discovered, raising an important question: how can one test for blood if it cannot be seen with the naked eye? It is not uncommon for an individual to attempt to conceal or clean up blood after committing a crime. Fortunately, the development of chemiluminescent and fluorescent-based presumptive tests such

as the luminol and fluorescein tests make it more challenging to definitively remove or mask blood on a surface.

Luminol ($C_8H_7N_3O_2$) is a chemical reagent and invaluable tool in the forensic toolbox for detecting trace amounts of blood that have either been cleaned up or concealed. Similar to the Kastle-Meyer and Hemastix[®] test, the luminol test also involves a chemical reaction between reagent and blood that yields a result visible to the naked eye. More specifically, the interaction between luminol and the iron group (Fe^{2+}) contained within hemoglobin results in the catalytic oxidation of luminol, which, in turn, will luminesce. Luminol is highly sensitive, as it can detect blood that has been diluted down to parts-per-million (ppm) concentrations and blood that has been painted over (James et al., 2014). However, it is important to note that the luminol test is not specific to blood, as bleaches containing sodium hypochlorite ($NaOCl$), metals enriched with iron II (Fe^{2+}), and plant peroxidases have all shown to yield false positives when exposed to luminol. The purpose of this experiment was to qualitatively measure the overall efficacy of luminol in terms of its ability to positively detect small bloodstains found on common household floor surfaces (wood, carpet, and tile) that have either been bleach cleaned with a 100% or 50% bleach solution; painted with two, four, six, eight, or 10 layers of solvent-based paint; or both bleach-cleaned and painted-concealed. According to research conducted by various academics, luminol can be detected on bloodstained surfaces that have either been cleaned with bleach, concealed by up to eight layers of water-based or solvent-based paint, as well as bloodstained surfaces that have been both cleaned with bleach and then painted over with water-based or solvent-based paint; therefore, a true positive luminol result should be attainable in all three experiments. By evaluating the efficacy of luminol in

various scenarios, the forensic science community will achieve a better understanding of the strengths and limitations of luminol, which may facilitate the development of new techniques or improvement of luminol or luminol-like methods for addressing these limitations.

Literature Review

Luminol Efficacy on Bleach-Cleaned Blood

Household bleach (or sodium hypochlorite) is a commonly employed reagent for removing blood; however, the use of luminol on surfaces suspected of containing blood has been shown to successfully prevent any attempts of concealment. While sodium hypochlorite can produce a false positive when luminol testing is performed, it is possible to differentiate the reaction between luminol and bleach and luminol and blood by observing the strength and length of chemiluminescent intensity. When luminol reacts with bleach, chemiluminescence is quick and likened to a burning sparkler or twinkling stars, whereas luminol reactions with blood are more intense and last several minutes (Brenzini & Pathak, 2018). According to Shaler (2012), flashes of light also indicate a false positive luminol reaction (as cited in Brenzini & Pathak, 2018).

Creamer and colleagues (2005) performed a quantitative study, which involved testing the chemiluminescent strength of luminol on bloodstains subjected to perpetual cleanings via water or bleach. In their experiment, glazed terracotta tile was sprayed with a hemoglobin solution and tested with luminol immediately or tested after one hour of drying. The luminol test was also conducted on tiles containing hemoglobin solution that had been cleaned with either water or bleach and then left to dry for zero, two, eight, or 16 hours. As expected, findings show that chemiluminescent intensity on hemoglobin stains cleaned with

water was inversely proportional to the number of cleaning steps and became undetectable to the naked eye after the 14th cleaning. They also found that hemoglobin stains allowed to dry for one hour were more difficult to remove and yielded a higher chemiluminescent intensity. Regarding hemoglobin stains cleaned with bleach, there was an initial spike in chemiluminescent intensity due to the increase in sodium hypochlorite reacting with the luminol molecules; however, the intensity level eventually returned to a level consistent with the positive detection of hemoglobin. The study also indicated that even though there was an initial interference from the bleach solution leading to chemiluminescent intensity levels higher than expected from detecting hemoglobin, this interference was negligible after eight hours, as evidenced by the fact that chemiluminescent intensity returned to a level consistent with the detection of hemoglobin (Creamer et al., 2005).

Luminol Efficacy on Painted-Concealed Blood

While it is common knowledge within the forensic science community that luminol can detect blood covered by paint, there appears to be a limited number of experimental studies regarding testing the chemiluminescent strength of luminol in such circumstances. Given the simplicity and ease of going down to a local hardware store, purchasing an inexpensive bucket of paint, and sacrificing a small amount of time to cover up any visible bloodstains left behind, it is curious to see the lack of experimental research on this subject. Bily and Maldonado (2006) found that a positive luminol reaction could occur on bloodstains that had been coated with a maximum of eight water-based paint layers (as cited in Brenzini & Pathak, 2018). However, Bily and Maldonado (2006) did note in their study that one limitation with luminol is that even though studies have shown luminol to be successful at

detecting blood underneath multiple layers of paint, the bloodstain patterns are difficult to recognize (as cited in Barrera et al., 2018). Laux et al. (2005) found that bloodstain patterns after luminol application appeared runny on non-absorbent surfaces such as non-textured linoleum or glass because of the smoothness and lack of porosity on the surface (as cited in Barni et al., 2007).

Nagesh and Ghosh (2017) performed a study on the efficacy of luminol on blood painted over but not cleaned. In their research, Ghosh and Nagesh (2017) applied chicken blood to concrete, wood, and metal surfaces, allowed the blood to dry on each surface for 24 hours, and then painted over each surface with one, two, or three layers of paint. White distemper paint was used on the cement surfaces, white enamel paint was used on the wood surfaces, and white automotive paint was used on the metal surfaces. The 1st layer of paint was allowed to dry for 30 minutes, while the 2nd and 3rd layers were immediately added after the 1st layer had dried. After applying the 2nd and 3rd layers, the paint was allowed to dry for 48 hours. Luminol was applied to each of the surfaces and then photographed six times over 50 days to document the chemiluminescent strength over time. From the results obtained from their study, Nagesh and Ghosh (2017) were able to make the following conclusions: (1) luminol was successful in detecting bloodstains underneath one, two, or three layers of paint, and on the concrete, wood, and metal surfaces; (2) a porous surface has a greater ability to retain a bloodstain and thus produce a stronger chemiluminescent intensity over a longer period of time, compared to a non-porous surface; (3) there was an inversely proportional correlation between chemiluminescent intensity and the number of paint layers for the metal and wood surface, but this relationship could not be seen with the cement surface; and (4) the amount of time that passed since concealment

did not affect chemiluminescent intensity on the concrete, wood, or metal surfaces.

Despite the successful results achieved by Nagesh and Ghosh (2017), there have been contrasting studies showing luminol to not be as effective at detecting blood concealed by paint. Vandenberg and Van Oorschot (2006) found that luminol testing on wood surfaces containing dried bloodstains that had been painted over with one layer of white acrylic paint or white, yellow, and green water-based paint did not test positive for the detection of blood; however, they did conclude that luminol can detect blood concealed by paint, so long as the bloodstain is not completely concealed. One solution to this problem is the use of BlueStar[®], which is a reagent similar to luminol. A study by Pettolina et al. (2017) found that BlueStar[®] was more effective than luminol at detecting blood concealed by multiple layers of paint (as cited in Barrera et al., 2018).

Luminol Efficacy on Bleach-Cleaned and Painted-Concealed Blood

Similar to the research conducted on the efficacy of luminol on painted over blood, there also appears to be a limited number of studies conducted on luminol use on blood cleaned with bleach and paint-concealed.

Brenzini and Pathak (2018) performed a comparison study that tested the strength of luminol on bloodstains that had been cleaned first and then painted over. In their experiment, ceramic tiles that either had or had not been cleaned via soap, water, or wet wipes and then left unpainted or painted via water-based or solvent-based paint were subjected to Kastle-Meyer (K-M) as well as luminol testing. The K-M test results indicated that positive results were achieved on tiles where the blood was in a dry or semi-dry state, regardless of the type of cleaning method

employed. Interestingly enough, K-M testing on fresh bloodstains led to negative results (no detection of blood). Brenzini and Pathak (2018) note that the cause for this may stem from the fact that dry and semi-dry bloodstains had more time to absorb into the tile before cleaning and would contain more hemoglobin K-M could then detect. Brenzini and Pathak (2018) also noted that both water-based and solvent-based paint yielded similar K-M results. Luminol testing was performed on dry, semi-dry, and fresh bloodstains that either had or had not been cleaned or and either not painted or painted over with one, two, three, four, five, six, or seven layers of water-based paint.

Regarding the dried bloodstains, a positive result was achieved on surfaces with up to four layers of paint, though the soap-cleaned tile was able to test positive up to six layers of paint. In each case, the intensity of chemiluminescence fell gradually with every additional layer of paint. Concerning semi-dry bloodstains, a positive result was achieved with up to six layers of paint, again with a gradual decline in chemiluminescent intensity for every additional layer. In regard to fresh bloodstains, the results were not as uniform. Soap-cleaned and water-cleaned stains yielded a positive result on up to five layers of paint, whereas the tiles cleaned with wet wipes yielded positive results on up to three layers of paint. Tiles that were not cleaned at all yielded a positive result on up to six layers of paint. Luminol testing was also performed on tiles that were either not painted or painted with one, two, three, or four layers of solvent-based paint. The results achieved were not as uniform when compared to the results achieved from the testing of tiles layered with water-based paint. While a positive result was achieved for all bloodstains with one layer of paint, the varying results from two, three, or four layers of paint made it difficult to determine any sort of trend.

Brenzini and Pathak (2018) also performed luminol testing on bleach-cleaned tiles. Tiles with no blood present that were cleaned with bleach exhibited a quick reaction. In contrast, tiles with blood present that had been cleaned with non-bleach methods and then subjected to water-based paint with a certain number of layers exhibited more intense chemiluminescence that lasted much longer. Interestingly enough, bloodstains that were cleaned with non-bleach methods and then subjected to solvent-based paint with a certain number of layers produced faded chemiluminescence that lasted only seconds rather than six-to-eight minutes, as seen in the water-based paint test.

Methods

Experiment One: Bleach-Cleaned Blood

The presence or absence of luminol, as well as the degree of chemiluminescence intensity, was qualitatively measured on wood, carpet, and tile surfaces containing a dried bloodstain that had been cleaned with either a concentrated bleach solution or a diluted bleach solution. Photographs were taken to provide visual documentation of the results.

Materials

Luminol; Synthetic blood; Clorox® Bleach; Allen + Roth 3” x 6” White Glazed Tile (2x); Exotic Hardwood 5.12” Tigris Bamboo Engineered Hardwood Flooring (2x); STAINMASTER Essentials LW186 Durable Touch II L011 Crossroads carpet sample; Medicine dropper; Canon EOS Rebel T3i DSLR; Kirkland Signature Create-A-Size Towel. Plastic Spray Bottle (2x).

Procedure

Using a clean medicine dropper, 10 drops of synthetic blood were applied to the two tiles, two wood, and two carpet samples. Each new drop of synthetic blood was directly applied on top of

the preceding drop to increase both the overall thickness and radius of the bloodstain. The synthetic blood was applied to the three sample surfaces in the same manner, to maintain uniformity within the experiment. Following application, the bloodstains on each of the three surfaces were left to air dry for one hour. The concentrated bleach solution was prepared by pouring Clorox[®] Bleach directly into a clean plastic spray bottle. The 50% bleach solution was prepared by adding 296 mL (10 fl. oz.) of Clorox[®] Bleach and 296 mL (10 fl. oz.) of distilled water into a separate clean plastic wash bottle. After drying for one hour, one of the tile, wood, and carpet samples was cleaned with the 100% bleach solution, while the other tile, wood, and carpet samples were cleaned with the 50% bleach solution. The cleaning method was uniform for each floor sample and entailed first wiping the bloodstain with a clean, dry paper towel, spraying the bleach solution three times onto the surface samples, scrubbing the surface of each floor sample with a clean, dry paper towel until the bloodstain was no longer visible to the naked eye, and drying the surface with a paper towel immediately after. Additionally, each floor surface was allowed to air dry for 24 hours in a windowless conference room with an ambient temperature between 21°C–22°C. This was done due to the assumption that at most crime scenes, suspected blood-containing surfaces have been cleaned days, weeks, months, or even years before CSIs arrive on the scene.

Experiment 2: Paint-Concealed Blood

The presence or absence of luminol, as well as the degree of chemiluminescent intensity, was qualitatively measured on wood, carpet, and tile surfaces containing a dried bloodstain that had been concealed with two, four, six, eight, or 10 layers of

solvent-based paint. Photographs were taken to provide visual documentation of the results.

Materials

Luminol; Synthetic blood; Allen + Roth 3" x 6" White Glazed Tile (2x) Exotic Hardwood 5.12" Tigris Bamboo Engineered Hardwood Flooring (2x); STAINMASTER Essentials LW186 Durable Touch II L011 Crossroads carpet sample; Valspar Ultra Semi-gloss Base A Latex Paint Medicine dropper; Canon EOS Rebel T3i DSLR.

Procedure

Using the same medicine dropper used in Experiment 1, 10 drops of synthetic blood were applied to eight tile, eight wood, and eight carpet samples. The bloodstains were allowed to air dry for one hour. Immediately thereafter, the bloodstains on each of the tile, wood, and carpet surfaces were painted with two, four, six, eight, or 10 layers of solvent-based paint. Each layer of paint was dried via a blow dryer set to the cool setting before adding a new layer. Once painted over with the appropriate number of layers, all floor surfaces were allowed to air dry for 24 hours in a windowless conference room with an ambient temperature between 21°C–22°C for the same reason as discussed in Experiment 1.

Experiment 3: Bleach-Cleaned and Paint-Concealed Blood

The presence or absence of luminol, as well as the degree of chemiluminescence intensity, was qualitatively measured on wood, carpet, and tile surfaces containing a dried bloodstain that had been first cleaned with either a concentrated bleach solution or diluted bleach solution and then concealed with two, four, six, eight, or 10 layers of solvent-based paint. Photographs were taken to provide visual documentation of the results.

Materials

Luminol; Synthetic blood; Allen + Roth 3" x 6" White Glazed Tile (2x); Exotic Hardwood 5.12" Tigris Bamboo Engineered Hardwood Flooring (2x); STAINMASTER Essentials LW186 Durable Touch II L011 Crossroads carpet sample; Valspar Ultra Semi-gloss Base A Latex Paint; Medicine dropper; Canon EOS Rebel T3i DSLR.

Procedure

With the same medicine dropper used in Experiments One and Two, and in the same fashion, 10 drops of synthetic blood were applied to 16 wood, carpet, and tile surfaces. The blood was allowed to air dry for one hour. After drying, eight of the wood, carpet, and tile samples were cleaned with the same 50% bleach solution used in Experiment One, while the other eight wood, carpet, and tile samples were cleaned with the same 100% bleach solution used in Experiment 1. All 16 surface samples were then painted with two, four, six, eight, or 10 layers of the same paint used in Experiment 2. Each layer of paint was dried via a blow dryer set to the cool setting before adding a new layer. The application of paint to each floor sample was conducted in the same manner as in Experiment 2. After cleaning and painting, all floor samples were allowed to air dry for 24 hours in a windowless conference room with an ambient temperature between 21°C–22°C.

Preparation and Visual Documentation of Luminol

After air-drying for 24 hours, all samples were moved to a windowless annex room where luminol testing could be adequately performed. The luminol solution was prepared by transferring the vial containing luminol powder into the separate activation fluid bottle and mixing thoroughly. The method by which luminol testing was photographed was modeled after an article in *Evidence Technology Magazine* written by Mark

Vecellio (2018) and was conducted as followed: (1) a pre-luminol photograph was taken of the floor sample with the lights on; (2) luminol was applied to the floor sample via a fine-mist sprayer with the lights off; (3) a positive luminol reaction was captured using a digital camera calibrated to capture chemiluminescence (f/5.6, 800 ISO, 10-second shutter speed, automatic white balance) and positioned at a 90° angle via a tripod. The photographs displayed in Figures 1, 2, and 3 were enhanced by increasing the exposure so that floor samples with faint chemiluminescence could be easily seen.

Results

Controls

A negative control test performed on a tile, wood, and carpet sample with no blood present on its surface did not test positive for chemiluminescence after applying luminol, indicating that there was nothing present on the samples that would test positive for luminol. A positive control test performed on a tile, wood, and carpet sample with blood cleaned with cold distilled water did test positive for luminol (Fig. 1), indicating that the luminol reagent was functioning properly.

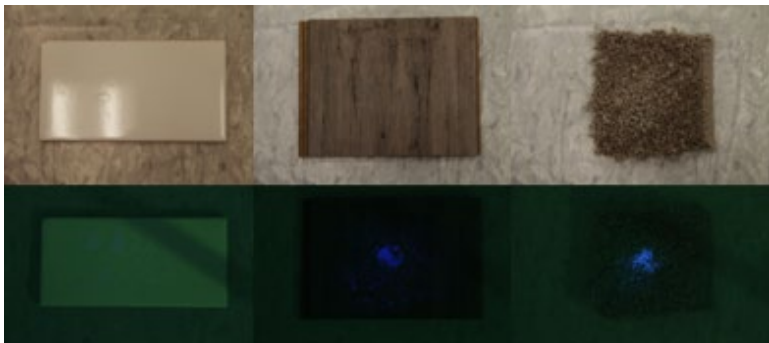


Figure 1. Positive Control

Experiment 1: Bleach-Cleaned Blood

Of the three floor samples cleaned with 50% bleach, only the tile and carpet samples tested positive for detecting blood (Table 1). The chemiluminescence emitted from the tile sample was limited to one small faint dot, whereas chemiluminescence emitted from the carpet sample was visible as a faint, mist-like pattern (Fig. 2A). Concerning the three floor samples cleaned with 100% bleach, only the wood and carpet samples tested positive for blood (Table 1). As with the tile sample cleaned with 50% bleach, chemiluminescence from the wood sample cleaned with 100% bleach was limited to two small, albeit slightly more intense, dots (Fig. 2B). Chemiluminescence from the carpet sample cleaned with 100% bleach had a similar pattern to the carpet sample cleaned with 50% bleach; however, the mist-like pattern for the carpet sample cleaned with 100% bleach was much more intense in its chemiluminescence (Fig. 2B).

Table 1

<i>Luminol Results on Bleach-Cleaned Blood</i>			
Bleach Concentration	Samples		
	Tile	Wood	Carpet
50%	Positive	Negative	Positive
100%	Negative	Positive	Positive

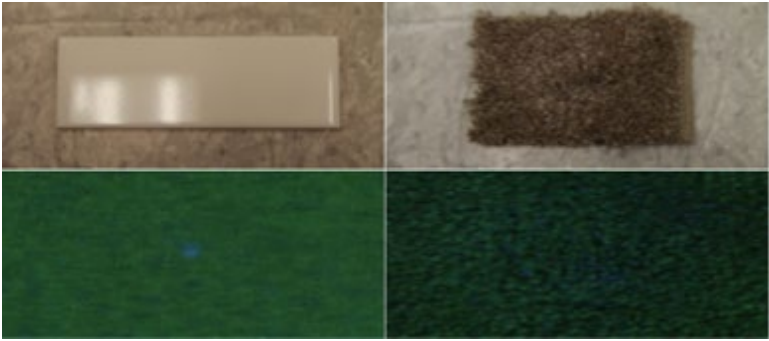


Figure 2A. Positive luminol results for surfaces cleaned with 50% bleach.

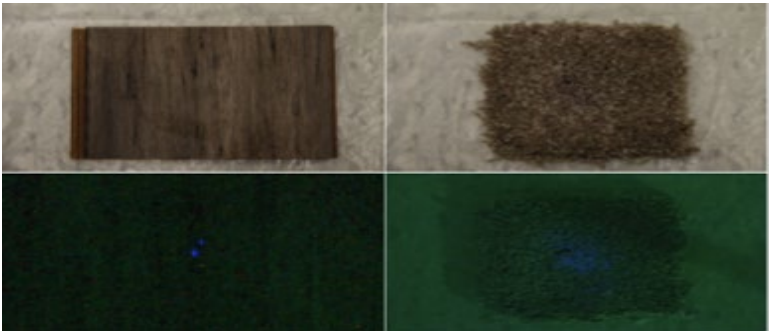


Figure 2B. Positive luminol results for surfaces cleaned with 100% bleach.

Experiment 2: Paint-Concealed Blood

As stated in Table 2, chemiluminescence could be seen on tile, wood, and carpet samples with two layers of paint; however, blood concealed by four, six, eight, or 10 layers of paint could only be positively detected on the carpet samples. Regarding chemiluminescent intensity, the tile and wood sample concealed by two layers of paint emitted faint chemiluminescence constrained to a small dot (Fig. 3A). Chemiluminescent intensity emitted from carpet samples concealed by four, six, eight, or 10

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layers of paint displayed varied results, with two and four layers emitting the higher intensities and six, eight, and 10 layers emitting lower intensities (Fig. 3A, 3B, 3C, 3D, and 3E).

Table 2

<i>Luminol Results on Paint Concealed Blood</i>			
No. of Paint Layers	Samples		
	Tile	Wood	Carpet
2	Positive	Positive	Positive
4	Negative	Negative	Positive
6	Negative	Negative	Positive
8	Negative	Negative	Positive
10	Negative	Negative	Positive

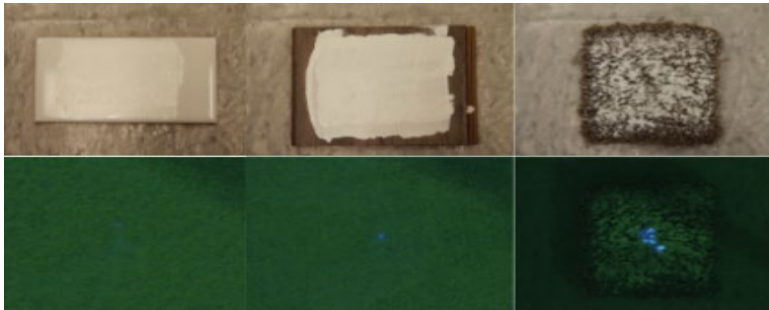


Figure 3A. Positive luminol results on two layers of solvent-based paint.

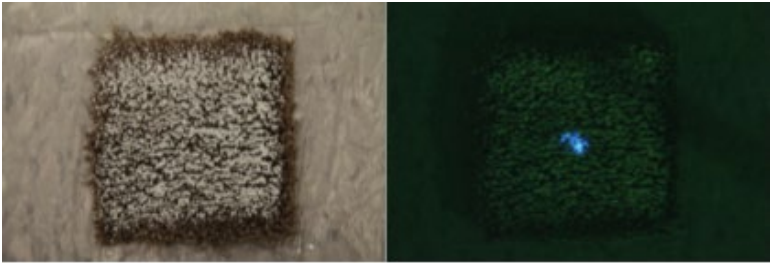


Figure 3B. Positive luminol results on four layers of solvent-based paint.



Figure 3C. Positive luminol results on six layers of solvent-based paint.



Figure 3D. Positive luminol results on eight layers of solvent-based paint.

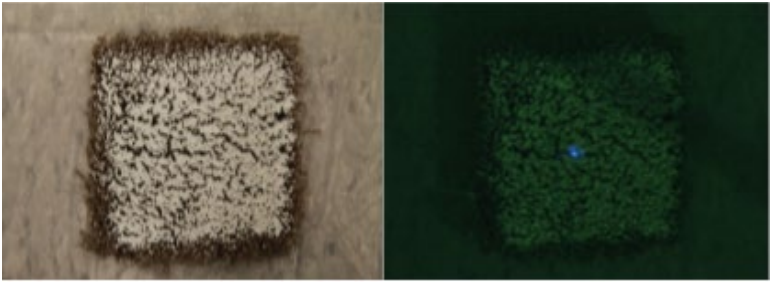


Figure 3E. Positive luminol results on ten layers of solvent-based paint.

Experiment 3: Bleach-Cleaned and Paint-Concealed Blood

All 10 floor samples cleaned with either 50% bleach or 100% bleach and then painted with 2, 4, 6, 8, or 10 layers of paint failed to produce lasting chemiluminescence from the luminol (Table 3); however, intermittent flashes of chemiluminescence were visible upon the immediate application of luminol to each floor sample due to the reaction between the luminol and the sodium hypochlorite found in the bleach. These flashes disappeared after a few seconds.

Table 3

<i>Luminol Results on Bleach-Cleaned and Paint-Concealed Blood</i>				
Bleach Concentration	No. Paint Layers	Samples		
		Tile	Wood	Carpet
50%	2	Negative	Negative	Negative
	4	Negative	Negative	Negative
	6	Negative	Negative	Negative
	8	Negative	Negative	Negative
	10	Negative	Negative	Negative
100%	2	Negative	Negative	Negative
	4	Negative	Negative	Negative
	6	Negative	Negative	Negative
	8	Negative	Negative	Negative
	10	Negative	Negative	Negative

Discussion & Conclusion

Experiment 1: Bleach-Cleaned Blood

As expected, the carpet samples could retain the highest amount of blood left behind after cleaning with both 50% and 100% bleach. The highly porous nature of the carpet allows blood to soak into its fibers, making it more difficult to clean blood out thoroughly. Interestingly, one would have expected the wood sample to also absorb enough of the blood to be detected by luminol after cleaning with both 50% bleach and 100% bleach due to the generally porous nature of wood; however, a positive result was obtained only for the wood sample cleaned with 100% bleach. Due to the inconsistencies in the tile and wood results, it is difficult to draw any solid conclusions with regards to the efficacy of luminol on either surface; however, with regards to the carpet samples, their results are consistent with the notion that carpet has a higher absorption rate than tile and wood. It is worth mentioning that any conclusions made about chemiluminescent intensity are difficult to draw for the tile, wood, and carpet samples, as it is difficult to determine any sort of trend.

Experiment 2: Paint-Concealed Blood

The positive results achieved with the tile, wood, and carpet sample at two layers were not surprising as studies such as the Nagesh and Ghosh (2017) study were able to detect blood concealed by up to three layers of paint, yielded similar results. The negative results achieved for the tile and carpet samples at four, six, eight, and 10 layers coincided with the Vandenberg and Van Oorschot (2006) study, which found that luminol testing on wood surfaces containing dried bloodstains that had been painted over with one layer of white acrylic paint or white, yellow, and green water-based paint did not test positive for the detection of

blood. With regards to the carpet samples, it is difficult to determine any sort of trend with chemiluminescence. From the photographs, chemiluminescent intensity is the strongest on bloodstains concealed by two and four layers, decreases in intensity at six layers, but increases slightly in intensity at eight and 10 layers. It makes sense that chemiluminescent intensity would decrease with each additional paint layer; however, the increase in intensity from four to six layers contradicts this. One possible reason for this may have to do with the possibility that the paint was not dry enough before adding a new layer, which would mean that the carpet samples may not have had the actual number of layers they were supposed to have.

Experiment 3: Bleach-Cleaned and Paint-Concealed Blood

The fact that luminol testing was negative for all tile and wood samples was not surprising, given the results from the other two experiments; however, it was surprising to find that all carpet samples turned up negative. Due to the lack of studies on luminol efficacy for blood on surfaces both bleach-cleaned and paint-concealed, it is difficult to compare these findings. The Brenzini and Pathak (2018) study used water, soap, and wet wipes to clean their blood samples before painting them over, whereas dilute and concentrated bleach solutions were used in this experiment. Possible reasons for why all samples tested negative may include the fact that the bloodstain was small and relatively fresh even after one hour of drying.

Future Considerations

From the results of the three experiments, it was concluded that luminol was not as effective at detecting small, fresh bloodstains on tile or wood surfaces that had been either painted over or bleach cleaned and then painted over but was effective at detecting small, fresh blood stains that had been cleaned with

bleach (50% and 100%) and painted with up to 10 layers of solvent-based paint. Given the uniform nature of the size of the bloodstain as well as the time allowed to air dry, future studies may want to explore the efficacy of luminol on bleach-cleaned, paint-concealed, and bleach-cleaned and paint-concealed blood, but as it relates to the size as well level of freshness of the bloodstain itself.

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