Article

Using Luminol to Detect Bloodstains Exposed to Fire, Heat, and Soot on Multiple Surfaces

Emma Akemann [1]
Lee C. Bushong [2]
Ward M. Jones [3]

Abstract: This article investigates the ability of luminol to detect bloodstains that have been exposed to fire, heat, soot, and water. Blood applied to drywall, appliance sheet metal, glazed tile, carpet, and wood was exposed to fire, followed by typical firefighter extinguishment. The results show that bloodstains on some surfaces that have been exposed to heat, fire, soot, or water may produce false negatives when tested with luminol.

Introduction

Previous research discusses the effects of heat on various surfaces to which blood is set [1, 2]. Heat contributes to the decomposition of blood. Other effects of fire, such as wind, smoke, or direct flame, may alter the boiling point of the blood [2]. In a study done by Brady et al. [1], blood that was subjected to high temperatures in a controlled environment showed a color change lighter than blood that was exposed to room temperatures [1]. This disagreed with the research of Kell et al. [2], who found that in a fire, bloodstains faded, but remained a normal brown color.

[1] Viterbo University, La Crosse, WI
[2] Florida A&M Univeristy, Tallahassee, FL
[3] Palmer College of Chiropractic, Davenport, IA

In previous studies [4–7], chemical enhancements, such as luminol, were tested to detect blood. However, an understanding of how these chemicals work to detect blood in soot-removed conditions was needed. This was necessary because fire may add debris to bloodstains, possibly making it more difficult for chemical enhancements to penetrate through to the stain. Bremmer et al. [8] attempted to find a new way of visualizing bloodstains without the use of chemicals. "Reflectance measurements were taken with a combination of a spectrograph (USB 4000; Ocean Optics, Duiven, the Netherlands), a tungsten-halogen light source (H-2000; Ocean Optics), and a noncontact probe (QR400-7-UV/BX; Ocean Optics)" [8]. This method was not highly useful because of the cost of the machinery. The typical chemicals (luminol and Bluestar) were effective in detecting small amounts of hemoglobin in blood [4]. Therefore, it was unnecessary to incorporate a new technique for forensic purposes, which worked equally as well, but was more expensive. Seashols et al. [6] compared hemascein to luminol to detect blood. Hemascein was found to yield poor results on common surfaces such as wood [6].

Two previous studies showed the effectiveness of retrieving and viewing blood evidence directly after being exposed to a fire [3, 7]. Both studies set a controlled fire and had firefighting personnel extinguish it [3, 7]. The resembled real-life efforts and the water damage that was done to the blood evidence provided real-life applications to the data. Kell et al. [3] used multiple patterns to compare different objects and surfaces. They noted all blood spatters were still visible; only their color and texture had changed [3]. One limitation of Kell et al.'s study was the length of time the structure burned–roughly 5 minutes. The blood was exposed to a lot of smoke and very high temperatures (900 °F), but the fire itself was not allowed to burn extensively [3]. The second experiment was done by Tontarski et al. [7], who constructed a small apartment. Blood was spattered throughout the set and the apartment was burned for 45 minutes until flashover conditions were reached. Firefighters extinguished the flames, and the samples were tested for the visibility of the blood and for DNA recovery. The use of luminol and Bluestar was successful in revealing missing stains (those that had disappeared because of the fire). Tontarski et al.'s study had a variety of surfaces for blood placement.

Overall, research on effective collection of blood evidence for arson cases was lacking, and although a limited number of studies demonstrated the effects of high heat on bloodstains [1, 2], there were only two that incorporated a live fire [7, 10]. Research has been done to prove which chemicals were best for identifying invisible stains [4]. However, only one tested these chemicals against burned bloodstains [5].

Research Questions

The objective of this study was to test the effect of luminol on bloodstains after exposure to fire and heat by testing for chemiluminescence on various surfaces. It was expected that blood evidence would be visible after soot was removed from the sample using the conventional method of a small brush [9] and that luminol would be effective in identifying the bloodstains through chemiluminescence [6]. Referencing the bloodstains' physical appearance, the pattern or outline of the stain was expected to remain unchanged [2], and the color would not be affected aside from a natural darkening when dried [1, 3]. Most importantly, it was hypothesized that the collection of blood evidence would still be deemed useful for the investigation of arson (especially considering the potential for evidence destruction [3, 7]). Therefore, the following research questions and null hypotheses were investigated:

ResQ1: Does fire or heat affect the chemiluminescence of luminol applied to blood on glazed wall tile?

(H_{01}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on glazed wall tile and there is no difference caused by fire or heat.

ResQ2: Does fire or heat affect the chemiluminescence of luminol applied to blood on oak trim?

(H_{02}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on oak trim and there is no difference caused by fire or heat.

ResQ3: Does fire or heat affect the chemiluminescence of luminol applied to blood on carpeting?

(H_{03}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on carpet and there is no difference caused by fire or heat.

ResQ4: Does fire or heat affect the chemiluminescence of luminol applied to blood on ceramic tile?

(H_{04}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on ceramic tile and there is no difference caused by fire or heat.

ResQ5: Does fire or heat and height affect the chemiluminescence of luminol applied to blood on ceramic tile?

(H_{05}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on ceramic tile and there is no difference caused by fire or heat and height.

ResQ6: Does fire or heat affect the chemiluminescence of luminol applied to blood on wooden floor laminate?

(H_{06}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on wooden floor laminate and there is no difference caused by fire or heat.

ResQ7: Does fire or heat affect the chemiluminescence of luminol applied to blood on drywall?

(H_{07}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on drywall and there is no difference caused by fire or heat.

ResQ8: Does fire or heat and height affect the chemiluminescence of luminol applied to blood on drywall?

(H_{08}) Fire or heat does not affect the chemiluminescence of luminol when applied to blood on drywall and there is no difference caused by heat and height.

ResQ9: Does fire or heat affect the chemiluminescence of luminol applied to blood on appliance metal?

(H_{09}) Fire or heat does not affect the chemiluminescence of luminol when applied to appliance metal and there is no difference caused by fire or heat.

Materials and Methods

Sample Collection and Support Construction

Surface samples were purchased from a local home improvement store. Structures were built using untreated pine 2" x 4" boards to hold samples at various heights. Structure 1 (Figure 1a) held seven samples of 4" x 4" glazed wall tiles (Color: VT10, Shape: 44HC1P4, Shade: 55501) at a height of 3' and seven 3" x 6" samples of prefinished golden oak trim (Base 18– 7/16"x 2") stained with Minwax Golden Oak 210B (417-1021) at a height of 2' 8". These samples were secured with a screw at the top center of the sample.

On the ground were two untreated pine 2" x 4" boards and two untreated pine 2" x 8" boards (Figure 1b) holding seven 6" x 6" carpet samples (Bridle Path, 12" nominal width–National–774-5116) stapled to the board, seven 6" x 6" ceramic tiles (Sanborn, wheat, 735-0702, 0710), seven 3" x 6"samples hickory natural stair nose (Great Lakes Wood Flooring, 3/4" x 48"– SKU: 742-2509–Color No: 16034), and seven 3" x 6" samples of wood laminate (Alloc Precision Flooring, 4243-SG). All samples on the ground, excluding the carpet, were secured with a screw at the top center of the sample. The carpet samples were stapled on all four sides to the support.

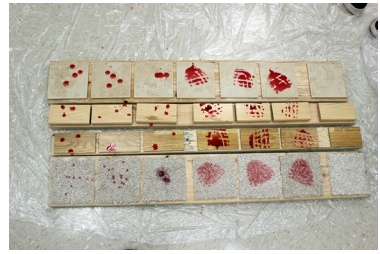
Structure 2, built of untreated pine 2" x 4" boards (Figure 1c) held ten 6" x 6" ceramic tiles (Sandborn, wheat, 735-0702, 0710) at a height of 7', a 8" x 4' gypsum wallboard handi-panel (5/8" thickness–131-1060) plank painted with interior paint (Behr Premium Plus Base, 2050, Interior eggshell enamel, 100% acrylic, mildew resistant finish) at 6', seven 6" x 6" ceramic tiles (Sanborn, wheat, 735-0702, 0710), 3' 5", two 8" x 4' gypsum wallboard handi-panel (5/8" thickness–131-1060) planks painted at 2' 10", and one 8' x 4' gypsum wallboard handi-panel (5/8" thickness–131-1060) plank painted with interior paint placed at ground level. All tile samples on Structure 2 were secured with a screw at the top center of the sample. The drywall planks were secured with screws underneath the tile boards at all four corners.

Two painted washing machine metal housings, one of which is shown in Figure 1d, were obtained from a local source. The housings were secured onto 2"x 4" boards to emulate the vertical surface of a washing machine.

Bloodstain Samples

Swine blood (from a local butcher) was collected in 10 mL vacutainer vials. The vials contained sodium-heparin to prevent clotting. On the same day that it was collected, the blood was spattered on various surfaces to replicate bloodstains normally found at a crime scene. The patterns that were created throughout the samples included cast-off (from approximately 4' away using a Chicago Cutlery knife), gravitational drops (using an autopipette from a height of 34 ½"), handprint transfer, footprint transfer (using a Nike size 9 tennis shoe), and one blood pool (formed by drips from a height of 34 ½"). Each sample was documented and left to dry overnight in the laboratory at 64 °F and 48 to 49% humidity. Before burning the samples, all samples

(a)



(b)

(c)

(d)

Figure 1

Blood application. (a) Structure 1 with wood and ceramic; (b) ground level samples; (c) Structure 2 with drywall and ceramic tiles; (d) washing machine structure.

were dry; however, some blood on the stair nose pieces did not adhere well to these samples.

The day after blood application, each frame and board was placed in the southwest corner of the local fire department's training center to burn (Figure 2).

Fire

The source of the fire consisted of wood pallets, shredded paper, and two furniture seat cushions (Figure 3). The materials were ignited by firefighting personnel with a handheld propane torch. Beginning three minutes later, temperature readings were taken using a thermal imaging camera by the trained firefighters through an opening in the access door to the burn room. Seven minutes after ignition, the tall support structure began to burn at which time fire suppression was initiated. The firefighting efforts were aimed at the fire through the opposing door to avoid washing the samples directly. The fire was extinguished in two minutes. After cooling down, the samples were removed and transported to the laboratory for processing. During the removal process, samples 6 (glazed wall tile) and 45 (ceramic tile) fell off the support and were retained for testing. As the drywall board, at the 6' level, was removed, it snapped into two pieces. The shorter side contained the cast-off blood pattern. The longer half contained the handprint transfer and control samples. The carpet samples curled; however, the stains were still visible. Smoke accumulation in the burn room resulted in the deposition of soot on all samples more than 2' above the floor level (Figure 4).

Figure 2

Set-up of burn room before ignition.

Figure 3
Source of the fire being ignited.

Figure 4
Photograph showing the soot covered down to about 2 feet.

Chemical Enhancement

Fresh working luminol solution was prepared using a 1:1:1:7 ratio of 0.4 mL sodium hydroxide, 0.176 mL hydrogen peroxide, 0.004 mL luminol [Sigma-Aldrich (97%), CAS #521-31-3], and water, respectively. Each sample that was covered in soot was brushed using a Sonia Kashuk duo-fiber fan brush to remove the soot while preserving the blood evidence. The solution was sprayed on each sample using a spray bottle. If the sample chemiluminesced, it was documented as a positive reaction. The foam board that was used as a dark background on the table was wiped down after each sample to avoid contamination. All samples were photographed before and after luminol treatment, shot on a tripod using a Canon EOS 70D SLR F/14 (150, 3200, 18-135@74 mm) with a length of exposure of 8 seconds. Samples with soot were photographed before and after soot removal as well as during luminol application. Photographs of the luminol attempts were taken in a completely dark setting to avoid light contamination.

Results

Burn Results

The tiles, drywall, and wood trim at the medium and high heights sustained the most damage from the fire. These samples were all charred and only the tiles were able to be brushed to remove the soot. The highest temperature reached during the fire was 863 °F, measured at the ceiling 4 minutes into the fire using a Drager thermal detector (UCF 6000) (Table 1). The smoke banked down to roughly 2' off the ground by the time the fire was extinguished (approximately 7 minutes after the fire was started). Bloodstain patterns could slightly be made out through the soot after the fire. Those samples without soot could still be seen clearly.

Level	Lowest Temperature	Highest Temperature
High	370 °	863 °F
Medium	250 °F	730 °F
Low	138 °F	447 °F

Table 1

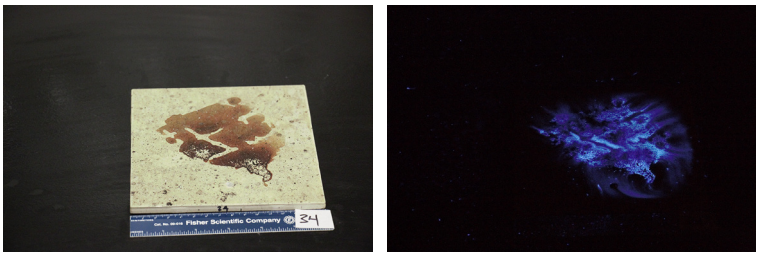
Maximum and minimum temperatures recorded.

Luminol Results

All samples were sprayed with luminol and photographed for reaction results. Samples on the ground included carpet, stair nose, wood laminate, ceramic tile, and drywall. These samples reached a maximum temperature of 447 °F and none were covered in soot. Of the six samples of wood laminate, five had a positive reaction with the luminol, meaning that they chemiluminesced. The same numbers were found with the stair nose and carpet samples. All six of the ground-level ceramic tiles yielded positive luminol results (Figure 5). All control samples were tested and yielded a negative response with the exception of the drilled hole of the ceramic control tile, which chemiluminesced. Both bloodstain patterns on the ground-level drywall chemiluminesced.

Samples at the midheight of about 3' included glazed tiles, wood trim, ceramic tiles, drywall, and the metal dryer sheets. All of the samples, except for wood trim, were brushed to remove soot, and all were negative in reaction with the luminol, as seen with the blood applied to the appliance metal (Figure 6a and 6c). Figures 6b and 6d reflect the lack of chemiluminescence upon application of luminol to the blood.

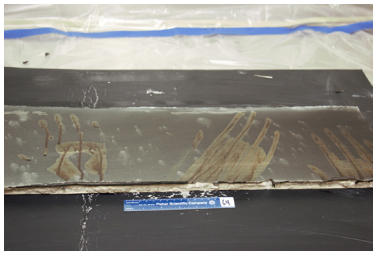
Samples at the high height of 7' included both ceramic tiles and drywall. The tile samples were brushed to remove soot and were negative in regard to luminol. The drywall was bubbled and flaking, so no attempt to remove soot was made. The drywall had a negative result to luminol (Figure 7).



(a) *(b)*

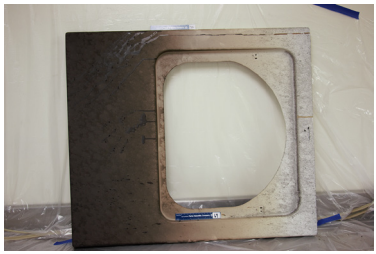
Figure 5

Example of ground-level ceramic tile shoe print after the burn (a) as well as after the luminol application (b).



(a)

(b)

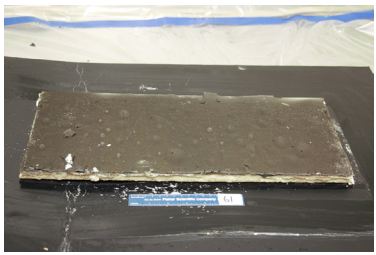


(c)

(d)

Figure 6

Photographs of mid-height samples after the burn (a, c) as well as after luminol application (b, d). The drywall shown in Figure 6 (a) is representative of all the drywall samples on this structure in that all six samples yielded negative luminol results, as did the appliance sheet metal 6 (c). This yield for 6 (c) is shown in Figure 6 (d).



(a)

(b)

Figure 7

Photographs of the high height drywall samples after the burn (a) as well as after luminol application (b). Both samples yielded negative results.

Discussion

Research Question 1 addressed the chemiluminescence of luminol on the surface of glazed wall tile and whether heat affected the chemical's ability to detect bloodstains. The glazed wall tile was affixed at a height of approximately 3' from the ground. The stains were brushed to remove soot and treated with luminol. The luminol did not chemiluminesce. Therefore, the null hypothesis (H_{01}) was rejected. A conclusion was reached that heat or fire prevented blood detection by luminol.

Research Question 2 addressed the chemiluminescence of luminol on oak trim and whether heat affected the chemical's ability to detect bloodstains. The oak trim was affixed at a burn height of approximately 2' 8" from the ground. After the burn, the stains were not brushed to remove soot because the grains likely would ruin any attempt to retrieve the bloodstain pattern. The bloodstains were treated with luminol. The luminol did not chemiluminesce. Therefore, the null hypothesis (H_{02}) was rejected. A conclusion was reached that heat or fire prevented blood detection by luminol.

Research Question 3 addressed the chemiluminescence of luminol on carpeting and whether heat affected the chemical's ability to detect bloodstains. The carpet was placed at ground level. After the burn, there did not appear to be any soot on the carpet. The carpet curled into a semiroll and became brittle. The bloodstains were treated with luminol. The luminol did cause a chemiluminesce reaction in five of the six samples. Therefore, the null hypothesis (H_{03}) was retained; a conclusion was made that fire or heat did not affect luminol's ability to react with the blood.

Research Question 4 addressed the chemiluminescence of luminol on ceramic tile (different from glazed tile studied in Question 1) and whether heat affected the chemical's ability to detect bloodstains. Ceramic tile was placed at burn heights at three different levels: ground level, 3' 6", and 7'. After the burn, the stains were brushed to remove soot and treated with luminol. The luminol chemiluminesced at the ground level. The luminol did not luminesce at the other levels. Therefore, the null hypothesis (H_{04}) was rejected. A conclusion was reached that heat or fire prevented blood detection by luminol. It is important to note that the blood at ground level was exposed to a maximum of 447 °F, whereas the other levels reached temperatures of 730 °F and 863 °F, respectively. Naturally, this leads to providing an answer for Research Question 5: Does height matter

when attempting to recover blood evidence using luminol? In this instance, the null hypothesis (H_{05}) was retained. The exact temperature that changes or damages blood was not determined, although an inference can be made that temperatures exceeding 447 °F become suspect.

Research Question 6 addressed the chemiluminescenced of luminol with blood on wood floor laminate and whether heat affected the chemical's ability to detect bloodstains. The laminate was placed at the floor level and when recovered, showed little signs of heat or soot damage. After the burn, the bloodstains were treated with luminol. The luminol chemiluminesced. Therefore, the null hypothesis (H_{06}) was retained; a conclusion was reached that heat or fire did not prevent blood detection by luminol.

Drywall was placed at differing levels in the burn area. It was placed at the floor level, 2' 10", and 6'. Research Question 7 looked to determine whether heat or fire prevented the luminol from reacting with the blood, and a presumptive answer is yes. Research Question 8 asked whether height played a difference in eliciting a response, and again, we answered yes. After the burn, the ground-level stains were not brushed because no interfering soot levels were noted. The 2' 10" -level sheets of drywall were brushed to reveal the bloodstain under the soot. The drywall placed at 7' was not brushed because the surface was extensively damaged by the fire and heat. The bloodstains were treated with luminol. A positive reaction was recorded for the stains applied to the ground-level drywall, and negative reactions were recorded for the others. Fire, heat, and height were found to be contributing factors. Therefore, we presumptively found that fire and heat did affect luminol's ability to react with blood even though we received ground-level positive results. Because we had a surface reaction to the blood, we retained the null hypothesis (H_{07}). Further, we rejected the null hypothesis (H_{08}) of Research Question 8, finding that height was a factor as an effect of greater heat within the confined area.

Research Question 9 addressed the chemiluminescence of luminol on appliance metal and whether heat affected the chemical's ability to detect bloodstains. The appliance metal sat on the ground and was about 3' tall. The stains were brushed to remove soot and treated with luminol.The luminol did not chemiluminesce. Therefore, the null hypothesis (H_{09}) was rejected. A conclusion was reached that heat or fire prevented blood detection by luminol.

Overall, an unexpected result was that most bloodstains could still be visualized after the fire. This supports the previous research of Kell et al. [3] Yet, a lot of samples yielded negative results from the luminol, which contradicts the findings of Tontarski et al. [7] Bloodstains may be recognizable after a fire, but presumptive field tests to prove the stain is indeed blood (e.g., luminol) may not always work. The higher the bloodstain was located in relationship to the floor, the less likely it was to react to the luminol. The bloodstains were more clearly seen after soot was brushed away, but the evidence still should not be disregarded because of a negative reaction to luminol.

All samples that were not covered with soot chemiluminesced, except the metal dryer sheets. The appliance sheet metal did not have a positive reaction to the luminol. This disagrees with the stovetop sample in the analysis by Tontarski et al. [7] The soot on the appliance sheet metal could not be brushed off and so one limitation to this study may have been the lack of another method of soot removal before testing this sample.

The most significant findings from the luminol tests were from the ceramic and glazed tiles at the medium and high heights. These tiles were covered in soot; when brushed away, the bloodstains could easily be interpreted. However, when luminol was applied to each tile, no chemiluminescence was observed, confirming the negative luminol results in one of the key studies looked at previously, in which a ceramic plate did not chemiluminesce on a bookshelf [7]. We believe that the glaze or finish on each tile melted at such a high temperature and then re-hardened over the bloodstains because we were able to directly see this effect. This effect was not noticed on the ground-level tiles, which chemiluminesced; however, they reached a significantly lower maximum temperature. A second explanation would be that a chemical in the top-coat of the tile may have masked the iron in the blood because of a chemical reaction. An example of this occurring was modeled in Bancirova's study where black and green tea mixed with a bloodstain decreased the chemiluminescence after exposure to luminol. The melted top-coat on our tile samples may have had the same effect in creating a false negative [10].

The samples on the ground were all positive. Perhaps evidence can be collected where minimal smoke and heat occur. Anywhere above these samples, the smoke had banked down onto the samples and they had reached heats of up to 863 °F (Table 1). These samples all yielded negative results.

The carpet samples chemiluminesced when exposed to the luminol, which is a new contribution to the scientific community when comparing the results of Tontarski et al.'s study where the carpet did not yield positive results [7]. This may have been because our carpet samples were stapled vertically to a 2" x 8" board that prevented the samples from absorbing large amounts of water from firefighting efforts. The samples were not installed onto the floor as real flooring would be. The smaller pieces curled up on the edges, possibly providing protection from the fire and firefighting efforts as well.

Lastly, drywall chemiluminesced when not burned by the heat and smoke. The samples above ground were charred and the paint was flaky. The ground sample that yielded a positive luminol result confirms results shown by Tontarski et al.'s study regarding painted drywall [7].

Conclusions

Many forensic services require a presumptive test on prospective bloodstains before collecting evidence or DNA samples. This research has shown that presumptive tests may not yield the correct analysis, as in a false negative. These findings may help investigators take note of potential evidence that would otherwise be disregarded when it comes to stains believed to be blood that do not test as such.

Further studies should be done to test presumptive tests other than luminol (e.g., a Hemastixtest strip that does not dilute the blood sample) on blood samples at higher heights and with soot contamination. Studies should also be done to replicate the burn in this study, because time and resources allowed for only one trial burn.

For further information, please contact:

Lee C. Bushong
Florida A&M University
1628 S. Martin Luther King Jr.
412B Perry-Paige
Tallahassee, FL 32307
lee.bushong@famu.edu

References

1. Brady, T.; Tigmo, J.; Graham, G. Extreme Temperature Effects on Bloodstain Pattern Analysis. *J. Bloodstain Pattern Analysis* **2002**, **18** (*2*), 3–20.
2. Larkin, B. A. J.; Banks, C. E. Preliminary Study on the Effect of Heated Surfaces upon Bloodstain Pattern Analysis. *J. For. Sci*. **2013**, *58* (5), 1289–1296.
3. Kell, M.; Kafarowski, E.; McGrory, J.; Sloan, M. The Effects of Fire on Bloodstains and DNA Recovery. Presented at IABPA Annual Training Conference, Corning, NY, 2006.
4. de Almeida, J. P.; Glesse, N.; Bonorino, C. Effect of Presumptive Tests Reagents on Human Blood Confirmatory Tests and DNA Analysis Using Real Time Polymerase Chain Reaction. *For. Sci. Int*. **2011**, *206* (1–3), 58–61.
5. Bilous, P.; McCombs, M.; Sparkman, M.; Sasaki, J. Detecting Burnt Bloodstain Samples with Light-Emitting Blood Enhancement Reagents. Presented at American Academy of Forensic Sciences, 62nd Annual Scientific Meeting, Seattle, WA, 2010.
6. Seashols, S. J.; Cross, H. D.; Shrader, D. L.; Rief, A. A Comparison of Chemical Enhancements for the Detection of Latent Blood. *J. For. Sci*. **2013**, *58* (1), 130–133.
7. Tontarski, K. L.; Hoskin, K. A.; Watkins, T. G.; Brun-Conti, L.; Michaud, A. L. Chemical Enhancement Techniques of Bloodstain Patterns and DNA Recovery after Fire Exposure. *J. For. Sci*. **2009**, *54* (1), 37–48.
8. Bremmer, R. H.; Edleman, G.; Vegter, T. D.; Bijvoets, T.; Aalders, M. C. G. Remote Spectroscopic Identification of Bloodstains. *J. For. Sci*. **2011**, *56* (6), 1471–1475.
9. Clutter, S. W.; Bailey, R.; Everly, J. C.; Mercer, K. The Use of Liquid Latex for Soot Removal from Fire Scenes and Attempted Fingerprint Development with Ninhydrin. *J. For. Sci*. **2009**, *54* (6), 1332–1335.
10. Bancirova, M. Black and Green Tea–Luminol False-negative Bloodstains. *Sci. Just*. **2012**, *52* (2), 102–105.