

RESEARCH ARTICLE

Enhancement of Bloodstains on Washed Clothing Using Luminol and LCV Reagents

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Introduction:

Luminol and LCV are commonly used reagents to develop latent bloodstains on evidence and at crime scenes. Luminol was first used to detect latent bloodstains in 1937 (1). Since that time the use of luminol has become very popular with many law enforcement agencies. The application of luminol creates a blue/green color chemiluminescence from its reaction with hemoglobin. Observation and subsequent documentation of latent bloodstain reactions with luminol require near to total darkness for best results. Leuco-crystal Violet (LCV) is another commonly used latent blood reagent for evidence and crime scenes. Bodziak (2) reports that the Federal Bureau of Investigation laboratory has utilized LCV since 1993. Like luminol, the application of LCV to latent bloodstains creates a catalytic reaction with hemoglobin. Unlike luminol, however, the LCV reaction is visible in normal lighting. LCV stains latent blood a dark purple to black color allowing for easy observation and documentation on light colored surfaces. Bodziak does caution that visible bloodstains on fabric are best processed with DAB or Amido Black reagents.

This research investigates the use of luminol and LCV to develop latent bloodstains from clothing, which has been washed with a commonly available cleaning product. A second aspect of this research was to test the use of the phenolphthalein as a presumptive blood test on the washed clothing items. A search of the major English language forensic journals and textbooks relating to bloodstain pattern analysis did not reveal any study that specifically examined the use of reagents on washed clothing. Quickenden et. al. (3) conducted research on the effectiveness of luminol in detecting washed bloodstains from automobile interiors. One interesting observation of their experiments was the conversion of hemoglobin to methemoglobin from increased heat in the motor vehicle following the deposition of blood. This resulted in and increased (enhanced) sensitivity of the luminol reaction. Not surprisingly, the authors discovered that repeated washings of interior surfaces decreased the sensitivity of the luminol reaction compared to non-washed surfaces. The authors did note, however, that the cleaning of carpet with a water and soap solution removed only the surface staining, leaving a strong presence within the foam padding of carpeting. Large quantitative differences in luminol reaction were observed between various carpet styles and commercial cleaning solutions however. Creamer et. al. (4) conducted research to determine the effect of the luminol reaction following the use of a known interfering catalyst (bleach) on washed items. The authors noted that luminol is highly sensitive, capable of detecting nanogram traces of blood. While their experiments were conducted on nonporous ceramic tiles, they observed that interference from bleach dissipated after approximately eight hours. DeHaan et. al. (5) also conducted sensitivity experiments with LCV on both porous and non-porous surfaces. Their research indicated LCV could detect blood at a dilution of 1:10,000, considerably less than luminol.

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Gifford (6) reported a case study in which bloodstains were found on the clothing of a male victim who had been discovered in water six days following his death. The author conducted experiments on bloodstained clothing in moving and stagnant water and found that bloodstains would not remain on the clothing after 30 minutes in moving water and not more than three hours in stagnant water. Certainly the action of the washing machine will dissipate blood at an even faster rate. Following his experiments, Gifford concluded that diffused blood still visible on the victim's wet or washed clothing was deposited after the clothing was removed from the water source (in that case a stream).

Materials and Testing Methods:

All experiments were conducted at the Arapahoe County Sheriff's Office Crime Laboratory in Centennial, Colorado in July of 2005. Whole horse blood obtained from a local veterinarian hospital was used for all experiments. Quickenden and Cooper (7) experimented with the luminol reaction using both human and bovine hemoglobin and found no significant difference in luminol reactions. Ten white colored Haynes brand "signature collection" 100% cotton undershirts were used for these experiments. The shirts had been worn for approximately 6-8 months prior to experimentation but had not previously been stained with blood. There was no visible discoloration or staining in the testing areas prior to the experiments. An eleventh shirt of the same condition was used as a control. The shirts were labeled #1-11 near the neckline with a black Sharpie brand marker (designations "B" and "F" for back and front). Three different types of bloodstain patterns were produced on both the front and back of each shirt (Figure 1).



Figure 1. Overall view of front of shirt # 5 with three bloodstain pattern locations.



Figure 2. Close view of shoe impression in blood on front of shirt.

Footwear impressions were produced by coating the outsole with a thin layer of blood, stepping on butcher paper to remove excess blood, and then stomping on the shirt (Figure 2). The projected bloodstain pattern (Figure 3) was created by forcing blood through a syringe onto the shirt. This created larger sized bloodstains with spinous processes. Misting bloodstain patterns were created by spraying the liquid blood through an aerosol sprayer (Figure 4). This created a very fine misting of blood characterized by a blood droplet diameter of less than 1mm. Paper inserts were used to prevent soak through from one side of the shirt to the other. The shirts were allowed to dry for one hour prior to washing. Washing and drying were done in stackable Frigidaire “Gallery” model units. Tide liquid laundry detergent with color safe bleach alternative was used for all washings. No other items were washed with the test shirts.



Figure 3. Close view of projected bloodstain pattern on front of shirt.



Figure 4. Close view of misted bloodstain pattern on front of shirt.

Shirts #1-5 were washed from one to five times with no drying cycles. Shirt #1 was washed a single time, shirt #2 two times, and so on. A new application of detergent was used for each wash cycle. Shirts #6-10 were washed in the same manner with a drying cycle of approximately one hour between each wash cycle. Shirt #6 had one wash and dry cycle, shirt #7 had two wash and dry cycles, and so on. Shirt #5 had five consecutive washing cycles with no drying of the shirt, while shirt #10 had a total of five alternating washing and drying cycles. The control shirt was subjected to a single wash cycle with detergent. After the final designated cycle each shirt was photographed in normal lighting. Each shirt showed a significant diffusion of blood staining over a large area that had a dull green colored appearance (Figure 5). A small sample of the green colored stain area (approx. 1cm²) was cut out from the sleeve of each shirt and tested with the phenolphthalein reagent. Samples were taken from the sleeve band area from each shirt where no direct bloodstaining had occurred while setting up the experiments. The shirts were then cut along their outer seams to

separate the front and back halves of the shirt. One half of the shirt was then processed with the luminol reagent while the other was processed with LCV. All photographs were taken with both a Nikon D100 and D2X Digital cameras. Good quality luminol exposures were shot at F3.5 between 15-25 sec.



Figure 5. Overall view of shirt # 5 showing dull green appearance after washing.

Discussion:

Immediate strong and positive phenolphthalein results were obtained on each shirt tested. Application of the phenolphthalein reagent, and subsequent hydrogen peroxide, were done directly on the fabric. This resulted in a “ring” appearance of the color reaction. In addition, similar sized areas were tested following the application of both luminol and LCV. Positive phenolphthalein reactions were achieved with both luminol and LCV treated shirts. All reagent and phenolphthalein testing on the control shirt was negative. LCV reactions on all shirts were immediate and pronounced. The reaction area appeared uniform and homogenous with no discernable or meaningful pattern recognition possible (Figure 6). Previous studies (8) have shown LCV to be a reliable latent blood reagent on unwashed clothing. The luminol reagent produced much better results on the tested clothing. Figures 7 and 8 show the luminol results on shirts #5 and #10. These shirts represent the materials that should show the lowest degree of luminol sensitivity due to their repeated cleanings. The projected bloodstain patterns were clearly visible and discernable in all ten

shirts tested (Figures 7-9). Conversely, the misting pattern was not discernable on any of the ten tested shirts. The footwear impressions were visible with luminol on shirts numbers # 2, #3, and #4 (Figure 9), and in plain view on shirts #7 and #8. The inconsistency regarding the presence or absence of footwear impressions may be due to varied degrees of blood volume and stomping pressure on the tested shirts. None of the footwear impressions contained sufficient detail for an identification with the known shoe, however, the physical size and design of the footwear was discernable in most cases.

The greenish colored bloodstain patterns on the shirts following the first wash cycle were likely the result of the bloodstains not being completely dried prior to washing. This staining presents several interesting challenges for the bloodstain pattern analyst. First and foremost, the visible and reagent staining bore little overall resemblance to the initial bloodstaining. While the projected and transfer (footwear) patterns could be seen in most cases, they were often intermingled with the “background” staining. Analysts who interpreted these diffused stains to be the result of any action other than washing would be incorrect in their analysis (in this specific case at least). In a similar fashion, the “background noise” created by this staining made identification of the initial stain areas more difficult using visible light. In the case of the misted blood it is unclear to the authors if the staining was actually present, albeit masked, by the additional staining caused by the washing cycles, or if it was completely destroyed by the washing cycle(s). The areas of projected blood and several of the footwear impressions were visible, but it was impossible for us to determine conclusively any sequence to the blood deposition on the “background” and “foreground”.



Figure 6. Overall view of shirt # 3 after LCV processing.



Figure 7. Overall view of luminol reaction on shirt # 5.



Figure 8. Overall view of luminol reaction on shirt # 10. impression



Figure 9. View of projected pattern and footwear on shirt # 3

Conclusion:

Investigators may be presented with washed clothing that is believed to contain bloodstains from violent acts such as homicide, assault, or sexual assault. Suspects, their associates, or victims may wash clothing following bloodshed, thereby destroying blood evidence and complicate the reconstruction process. Diluted bloodstains resulting from machine washing may not be visible especially on dark colored clothing. In such cases, the use of a chemical reagent may be the only acceptable method for developing latent bloodstains. Regardless of which reagent is used to visualize latent bloodstains, analysts should use caution when interpreting diffused or diluted bloodstain patterns occurring over a large area of the clothing in question. This level of saturation may be the result of the washing process and may not relate to any one specific blood letting event. This research supports the use of luminol as an effective reagent to visualize latent bloodstain patterns on washed clothing. LCV, while an effective blood reagent on many washed and unwashed surfaces (personal observations of the senior author), did not yield acceptable results in this study. Analysts are cautioned in using LCV on washed clothing or other washed porous items. Furthermore, our research indicates that phenolphthalein will yield presumptively positive results on washed clothing, even after application of these two chemical reagents. Analysts are encouraged to report similar testing results to aid in defining the sensitivity and proper usage parameters of LCV and luminol on cleaned porous and non-porous surfaces.

References:

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