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Detection of Blood on Water-Repellent Cottons Before and After Laundering

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Detection of Blood on Water-Repellent Cottons Before and After Laundering

By

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THESIS

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Abstract

When a perpetrator attacks an individual, trace evidence in the form of blood may become attached to a worn garment. However, if the perpetrator wears a garment containing water-repellent properties, blood evidence may not be apparent. The blood evidence may become further undetected if the fabric is laundered in a washing machine, which is what perpetrators may try to get rid of incriminating evidence.

To model this scenario, I conducted an experiment to determine if synthetic blood on water-repellent-coated and uncoated cotton and cotton/polyester blend fabric samples can be detected using visual examinations. These examinations included four presumptive blood tests – Kastle-Meyer (KM) Test, Luminol, Leucocrystal Violet (LCV), and Hemascein – that were used on samples before and after being laundered.

The results of the tests varied from sample to sample, but most were successful in detecting the blood on the coated and uncoated fabric samples before and after laundering. All tests performed exceptionally for all coated and uncoated samples before being laundered by indicating a positive result for the detection of blood. For the post-laundered samples, however, Luminol and Hemascein were the only tests that showed positive results for blood on both coated and uncoated cotton and cotton/polyester blend fabric samples. The KM test and LCV tests that were either negative or inconclusive on both the coated and uncoated samples after being laundered. Further testing should be conducted in different environments and among different researchers to achieve a greater sample population for more precise results. If further results are consistent with these findings, caution should be taken when utilizing these tests to analyze potential evidence in criminal cases.

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Figures and Tables

Chapter 1: Introduction

Cotton

Cotton is a natural staple fiber that comes from the seed hair of a plant. It grows into a fruit and is then salted to extract the long white fibers. The fibers typically have rounded cross-sections when cut before being processed. However, once they are dried, scoured, and bleached through fiber processing, the fibers become hollow and collapse onto themselves, forming kidney-bean shaped cross sections. Regardless of its shape, the cotton fibers are made up of cellulose structures, a carbohydrate polymer that has many hydroxyl groups in the cellulose chains. These hydroxyl groups are what make cotton fibers hydrophilic, attracting moisture, and absorptive to water-soluble substances. With modern innovations and ideas, cotton fibers and fabrics have been a topic of much research.

The hydrophilic nature of cotton fibers has a great advantage for the use in consumer textiles; however, such a property also elicits disadvantages as well. The prospects of hydrophobic cottons as a means to repel fluids are a great way of showing how cotton fibers can be proliferated in technical clothing materials. Research conducted by Wadsworth & Allen (1998), Yaghoubidoust & Salimi (2019), Wadsworth & Tsai (2005), Nguyen (2018), and Yuen et al (2005) has explored the addition of functions that can repel biological fluids and bacteria for personal protective equipment (PPE) uses while sustaining the breathability of cotton garments. These textiles are designed to be water repellent, antimicrobial, antibacterial, and antiplatelet hydrophobic cotton fabrics that retain the breathability of cotton and can be used in the hospital and research settings as a barrier between a person and biological fluids they may encounter.[1]

[1] L. C. Wadsworth and H. C. Allen, Jr, "Development of Highly Breathable and Effective Blood/Viral Barrier Laminates of Microporous Films, Staple Fibers and Nonwovens," *Journal of Coated Fabrics* 28 (1998): 12-28; F. Yaghoubidoust and E. Salimi, "Antibacterial and Antiplatelet Properties of Octyltrichlorosilane-modified Cotton Fabrics." *Fibers and Polymers*, 0 (2019): 1-5; L. C. Wadsworth and P. P. Tsai, "Enhancement of Cotton Containing

At this time, the water-repellent functions on fabrics are defined as blocking water while maintaining the breathability of the fabrics, which can be achieved in surface modification of cotton fibers with hydrophobic chemicals or coating the fabric with a layer of thin polymers. The surface treatments of fibers in cotton fabrics could be done in commercial finishing processes to achieve durable performance or by spray coating to achieve temporary functions, which could be washed off and resprayed. The spray coating process is doable by consumers or companies. Examples include couches, carpets, and outdoor furniture, as well as apparels. For wearable fabrics, this concept can be seen in outdoor clothing manufactured for outdoor activities, such as hiking, biking, camping, and the like, or for PPE in hospital and scientific settings to lessen the amount of soiling on the garment.

Water repellent agents used in textile treatments are hydrophobic chemicals, including fluorocarbon, silicones, and others. Fluorocarbons were widely employed, with excellent water and soil repellency, but these types of treatments are being reassessed due to their persistence in and potential harm to the environment. With increases in technological advances, textile industries are progressively figuring out ways to incorporate these products into everyday life, while keeping sustainability in mind. While wear and tear on clothing is inevitable over time, some companies try to combat this by reimagining how clothing is made today so that it can last tomorrow. While the prospects of a stain-resistant clothing or biological-fluid-repelling PPE sounds appealing and helpful to concerned individuals, the hydrophobicity of such garments could become problematic within the forensic discipline.

Barrier Fabrics with Breathable Films and Protective Finishes for Safety from Biological Threats." 2005 Beltwide Cotton Conferences, Cotton Inc., New Orleans, LA. Conference Presentation; T. Nguyen, "Multifunctional Smart Textiles: Influences of Hydrophobic Additional Finished on Antimicrobial Treated Cotton Fabric." 2018 4th International Conference on Green Technology and Sustainable Development (GTSD), Aconf Conference Solution Professional, Hoi Chi Minh City, Vietnam. Conference Presentation; C. W. M. Yuen, Y. Li, K. S. Ku, C. M. Mak, and C. W. Kan, "Experimental study on fabric water repellency using nanotechnology." *AATCC Review* 5, 8 (2005): 41-45.

Water-Repellent Textiles with Blood

Previous studies relating to water-repellent/hydrophobic textiles have examined different aspects that might affect the analysis of crime scene evidence. Acrylic and polyester have been tested with steam thermography and thermal or infrared cameras to determine the presence of blood patterns.[2] Belliveau et al. (2016) used steam thermography to look at minute details of fingerprint ridge patterns that transferred rat blood onto three fabric types: acrylic, polyester, and cotton. They concluded that for the more hydrophobic fabrics acrylic and polyester, the detailed prints were readily visible during the heating-up and cooling-down stages of their steam experiment. The more hydrophilic cotton fabric, however, did not show detailed results.[3] This was most likely due to the hydrophilic properties of cotton and good absorbance to the water-soluble properties of blood thus obscuring any fine details that could have persisted.

Similarly, O'Brien et al. (2015) used thermal imaging chemical contrast to look at letters and handprints on acrylic and polyester fabrics exposed to steam. They theorized that the "hydrophilic nature of blood proteins … would absorb more water than the surrounding fabric," thus creating a high contrast between the stain and the fabric when steamed. Their theory was supported by the results in the steaming technique, coupled with thermal imaging, producing "immediate and strong [thermal-imaging chemical contrast], even when the blood stain [was] diluted 100 times or more." These tests were successful in visually producing blood patterns on textiles, even after leaving the blood on the fabrics after long periods of time (eight months to three

[2] R. G. Belliveau, S. A. DeJong, B. M. Cassidy, Z. Lu, S. L. Morgan, and M. L. Myrick, "Ridge patterns of blood-transferred simulated fingerprints observed on fabrics via steam thermography." *Forensic Chemistry* 1, (2016): 74-77; W. L. O'Brien, N. D. Boltin, Z. Lu, B. M. Cassidy, R. G. Belliveau, E. J. Straub, S. A. DeHong, S. L. Morgan, and M. L. Myrick, "Chemical contrast observed in thermal images of blood-stained fabrics exposed to steam." *Royal Society of Chemistry, 140*, (2015): 6222-6225.
[3] Belliveau et al., "Ridge Patterns of Blood."

years). These studies, however, showed results for hydrophobic fabrics acrylic and polyester, while the cotton results were either dismissed or not tested.[4]

Another study looked at some existing forensic techniques to enhance bloody footwear impressions before and after laundering on a variety of fabrics. Farrugia et al. studied how different peroxidase reagents could enhance the visibility of a bloody footwear impression on different textiles. They tested peroxidase reagents, leucocrystal violet (LCV), Leucomalachite green (LMG), fluorescein, and luminol on white cotton, black cotton, patterned cotton, white polyester taffeta, black polyester taffeta, white nylon/lycra blend, black nylon/lycra blend, blue denim, and brown bovine leather. A shoe, attached to a shoe-imprinting rig, was used to produce bloody impressions onto the different fabrics and were left for seven days. Afterward, the fabric samples were tested with the reagents, washed, and then left to air dry overnight. They found that before washing, luminol had the most optimal results overall and was the only test to detect impressions on denim. On the other hand, LCV and LMG were found to be only suitable for the blood impressions on all light-colored fabrics. Unfortunately, after washing the material, the researchers found that none of the tests performed well.[5]

Continued research with presumptive blood tests to detect blood on surfaces has been done by Seashols et. al., who used blood to test the limits of detection of three presumptive tests. They examined how well luminol, Bluestar, and Hemascein could detect dilute solutions of blood on different surfaces including linoleum, wood, cotton fabric, and nylon fabric. While the luminol

[4] O'Brien et al., "Chemical Contrast Observed in Thermal Images."
[5] K. Farrugia, K. A. Savage, H. Bandey, T. Ciuksza, and N. N. Daeid, "Chemical enhancement of footwear impressions in blood on fabric — Part 2: Peroxidase reagents." *Science and Justice*, *51* (2011): 110–121.

and Bluestar performed similarly across all the tested surfaces, Hemascein detection levels yielded the highest on fabrics, but the lowest on wood.[6]

Taking inspiration from these scholars, I examine how well four common presumptive forensic blood tests behave on a newer conceptual fabric that may one day become more widely utilized in society.

Forensic Presumptive Tests

The Kastle-Meyer (KM) reagent is highly sensitive for detecting the presence of blood.[7] For the KM reagent to change color, the heme from hemoglobin in blood must be present. When the heme is coupled with hydrogen peroxide, it creates a peroxidase-like reaction, breaking down the hydrogen peroxide and oxidizing the phenolphthalein in the KM reagent. This changes the reagent from colorless to a pink color, which is indicative of a positive result for the presence of blood.[8]

[6] S. J. Seashols, H. D. Cross, D. L. Shrader, and A. Rief, "A Comparison of Chemical Enhancements for the Detection of Latent Blood." *Journal of Forensic Science* 58, 1 (2013): 130-133.

[7] A. H. Lowe, J. Bagg, F. J. T. Burke, D. MacKenzie, and S. McHugh, "A study of blood contamination of siqveland matrix bands." *British Dental Journal*, 192 (2002): 43-45; J. Sloots, W. Lalonde, B. Reid, and J. Millman, "Kastle-meyer blood test reagents are deleterious to DNA." *Forensic Science Journal*, 281 (2017): 141-146; J. L. Webb, J. I. Creamer, and T. I. Quickenden, "A comparison of the presumptive luminol test for blood with four non-chemiluminescent forensic techniques." *Luminescence*, 21 (2006): 214-220.

[8] Lowe et. al, "A study of blood contamination"; M. Cox "A study of the sensitivity and specificity of four presumptive tests for blood." *Journal of Forensic Sciences* 36, 5 (1991): 1503-1511; Sloots et al., "Kastle-Meyer blood test reagents"; J. J. Glaister, "The kastle-meyer test for the detection of blood: considered from the medico-legal aspect." *The British Medical Journal* 1, 3406 (1926): 650-652; S. Mushtaq, N. Rasool, and S. Firiyal, "Detection of dry bloodstains on different fabrics after washing with commercially available detergents." *Australian Journal of Forensic Sciences* 48, 1 (2016): 87-94; S. M. S. M. Daud, and S. Sundram, "Identification of bloodstains on different fabrics after washing in commonly used detergent in malaysia." Journal of Management and Science 17, 1 (2019): 57-65; R. I. B. Fonseca, E. L. Ricci, H. de Souza Spinosa, M. M. Bernardi, G. R. de AbreuP. A. F. Waziry, M. A. Nicoletti, S. R. Ambrosio, I. P. se Arujo, J. W. P. Munoz, and A. R. Fukushima, "Actual trends in the use of the kastle-meyer test: applications in different species and verification of the limit of detection of sensitivity and vestigiality." *Journal of Dairy, Veterinary & Animal Research* 8, 4 (2019): 166-170; J. L. Webb, J. I. Creamer, and T. I. Quickenden, "A comparison of the presumptive luminol test for blood with four non-chemiluminescent forensic techniques." *Luminescence*, 21 (2006): 214-220.

Luminol is a peroxidase reagent, meaning it has heme-reacting chemicals, and typically contains hydrogen peroxide as its oxidizing agent.[9] When this reagent is introduced to hemoglobin/blood, the heme group in the blood catalyzes the oxidation of the hydrogen peroxide, thus decomposing the luminol solution.[10] Generally, this reaction is described as the "oxidation and excitation of luminol resulting in the excited state dianion intermediate, 3-aminophthalate, that upon return to the ground state emits a broad spectrum of light centered around 425 nm."[11] This means that luminol starts at the ground state and moves to the excited state (when oxidized by the heme group). Upon returning to the ground state from the excited state, it emits the chemiluminescent light that can be observed in darkness.[12] This procedure should be done under minimal to no light for best results. As a forensic presumptive technique, it is shown to still be able to detect blood after washing a garment and does not negatively affect DNA samples in the blood so it can then be swabbed for further analysis.[13]

[9] Belliveau et al., "Ridge patterns of blood-transferred"; Webb, Creamer, and Quickenden, "A comparison of the presumptive luminol test."
[10] Farrugia et al., "Chemical enhancement of footwear"; B. M. Cassidy, Z. Lu, J. P. Martin, S. K. Tazik, K. W. Kellogg, S. A. DeJong, E. O. Belliveau, K. E. Kilgore, S. M. Ervin, M. Meece-Rayle, A. M. Abraham, M. L. Myrick, and S. L. Morgan, "A quantitative method for determining a representative detection limit of the forensic luminol test for latent bloodstains." *Forensic Science International*, *278* (2017): 396-403; P. Khan, D. Idress, M. A. Moxley, J. A. Corbett, F. Ahmad, G. von Figura, W. S. Sly, A. Waheed, and M. I. Hassan, "Luminol-based chemiluminescent signals: clinical and non-clinical application and future uses." *Applied Biochemistry Biotechnology*, 173 (2014): 333-355; J. Finnis, J. Lewis, and A. Davidson, "Comparison of methods for visualizing blood on dark surfaces." *Science and Justice*, 53 (2013): 178-186; D. Howard, J. Chaseling, and K. Wright, "Detection of blood on clothing laundered with sodium percarbonate." *Forensic Science International* 302, 109885 (2019): 1-7.
[11] Cassidy et al., "A quantitative method."
[12] Cassidy et al., "A quantitative method"; Khan et al., "Luminol-based chemiluminescent"; Finnis, Lewis, and Davidson, "Comparison of methods."
[13] Belliveau et al., "Ridge patterns of blood-transferred"; M. M. Gupta, V. Saran, M. K. Mishra, and A. K. Gupta, "Examination of traces of blood stains on different fabrics after washing." *Journal of All Research Education and Scientific Methods* 4, 6 (2016): 204-209; S. S. Tobe, N. Watson, and N. N. Daeid, "Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular-weight DNA." *Journal of Forensic Science*, 52 (2007): 102-109.

Leucocrystal violet (LCV), also known as gentian violet, is a highly specific stain that reacts with heme in blood.[14] When the hydrogen peroxide-containing LCV reagent is used in the presence of blood, it will change from a clear colorless solution to a purple/violet color.[15] This occurs because LCV is the completely reduced form of crystal violet (CV).[16] There is a dimethyl group on LCV that delocalizes a positive charge in the presence of heme and hydrogen peroxide, changing the once clear, colorless LCV into a deep violet color CV.[17]

Hemascein is a relatively new preliminary blood testing kit, developed by Abacus Diagnostics, which uses fluorescein as its main reagent. Like luminol, fluorescein is a peroxidase reagent. When combined with water, the fluorescein is reduced to fluorescin. This fluorescin solution should then be sprayed lightly onto a suspected surface, and then a lightly sprayed lightly application of hydrogen peroxide should follow. When these two solutions come into contact with the heme group in hemoglobin, it emits a green chemiluminescence.[18] Unlike luminol, chemiluminescence should be viewed using an orange/red filter accompanied by an alternative light source (ALS) in the 425-485 nm wavelength range.[19]

[14] N. Praska, and G. Langenburg, "Reactions of latent prints exposed to blood." *Forensic Science International* 224 (2013): 51-58; D. Petretei, and M. Angyal, "Recovering bloody fingerprints from skin." *Journal of Forensic Identification* 65, 5 (2015): 813-827.
[15] Praska and Langenburg, "Reactions of latent prints"; Petretei and Angyal, "Recovering bloody fingerprints"; L. Spence, and G. Asmussen, "Spectral enhancement of leucocrystal violet treated footwear impression evidence in blood." *Forensic Science International*, 132 (2003): 117-124; W. M. Bodziak, "Use of leuco crystal violet to enhance shoe prints in blood." *Forensic Science International 82* (1996): 45–52.
[16] Spence and Asmussen, "Spectral enhancement"; Petretei and Angyal, "Recovering bloody fingerprints."
[17] Farrugia et al., "Chemical enhancement of footwear."
[18] Seashols et al., "A Comparison of Chemical Enhancements"; Finnis, Lewis, and Davidson, "Comparison of methods"; T. Lowis, K. Leslie, L. E. Barksdale, D. O. Carter, "Determining the sensitivity and reliability of Hemascein." *Journal of Forensic Identification* 62, 3 (2011): 204-214.
[19] Seashols et. al., "A Comparison of Chemical Enhancements"; Finnis, Lewis, and Davidson, "Comparison of methods."

Current Study

The current study evaluates whether the Kastle-Meyer Test, Luminol, LCV, and Hemascein can detect the presence of blood on cotton and cotton/polyester blend fabrics spray coated or uncoated before and after laundering. This study seeks to assess whether these common presumptive tests can perform and accurately detect the presence of blood on the fabrics with water repellent functions.

Chapter 2: The Experiment

To carry out this experiment, different procedures were adapted and conducted on the fabric samples in preparation for their respective presumptive testing methods. The following parts describe how each procedure was executed. All photographs/videos in this study were taken using the author's personal cell phone, a Samsung S22 Ultra, with the camera specs shown in Table 2.1.

Table 2.1 Rear camera specifications of the Samsung S22 Ultra used in taking all the photographs presented in this study. Specifications taken from the Official Samsung Website.[20]

Rear - Ultra Wide	12MP F2.2 [Dual Pixel AF], FOV 120°, 1/2.55," 1.4µm
Rear - Wide Angle	108MP F1.8 [PDAF], OIS, FOV 85°, 1/1.33," 0.8µm with Adaptive Pixel
Rear - Telephoto 1	10MP F2.4 [3x, Dual Pixel AF], OIS, FOV 36°, 1/3.52," 1.12µm
Rear - Telephoto 2	10MP F4.9 [10x, Dual Pixel AF], OIS, FOV 11°, 1/3.52," 1.12µm
Rear - Space Zoom	3x, 10x Dual Optical Zoom Super Resolution Zoom up to 100x

[20] "Camera Specs & Features - Galaxy S22 Ultra, S22+ & S22 5G." *Samsung Ie*, www.samsung.com/ie/support/mobile-devices/check-out-the-new-camera-functions-of-the-galaxy-s22-series/#:~:text=Check%20out%20the%20Galaxy%20S22%20Series%20Camera%20overview

Part 1: The Cutting

Two types of fabric, 100% Cotton Knit and Cotton/Polyester blend, were obtained from the United States Department of Agriculture Southern Regional Research Center and Joann's Fabrics, respectively. Once obtained, the fabrics were cut into thirty-six (36) 2-inch squares and were weighed individually, and photographs and RGB values were taken from each. The samples were then divided into nine (9) different sets.

Of the total thirty-six (36) fabric samples, nine (9) cotton squares and nine (9) cotton/polyester blend squares were divided and placed into labeled, resealable bags. After cutting, the weight of the samples was taken to ensure approximate likeness in size.

The rest of the nine (9) cotton squares and nine (9) cotton/polyester blend samples were spray-coated with a 3M brand Scotchgard Fabric Water Shield (SG), to obtain the water-repellent function.

Part II: The Coating

Nine (9) cotton squares were placed individually on nine (9) watch glasses in preparation for the water-repellent spray coating. Application of the coating was carried out by following the instructions given on the aerosol can of the Scotchgard, which is made from trade secret amounts of hydrotreated light petroleum distillates, petroleum gases, proprietary silicone mixture, and proprietary resin.[21] The cans were shaken for about thirty (30) seconds. The cans were then held about six (6) inches away from the fabric samples and sprayed in a slow, sweeping motion over each. The samples were flipped and sprayed using the same method on the backside of the fabric

[21] "Scotchgard Fabric Water Shield." 3M Safety Data Sheet, 2023, https://multimedia.3m.com/mws/mediawebserver?mwsId=SSSSSuUn_zu8l00xNx_SOxtvov70k17zHvu9lxtD7SSSSSS-

samples. The freshly coated samples, except for the samples in the “Sample Set: to UC Davis” set, can be seen in Figures 2.2.1 and 2.2.2.

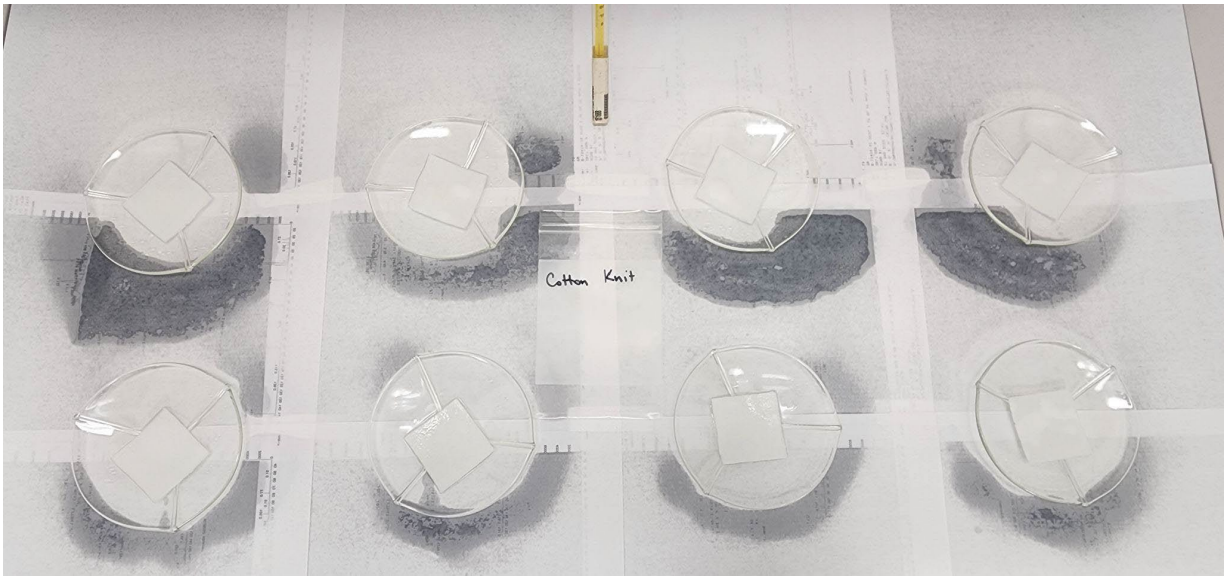

Fig. 2.2.1 Cotton samples spray coated with Scotchgard

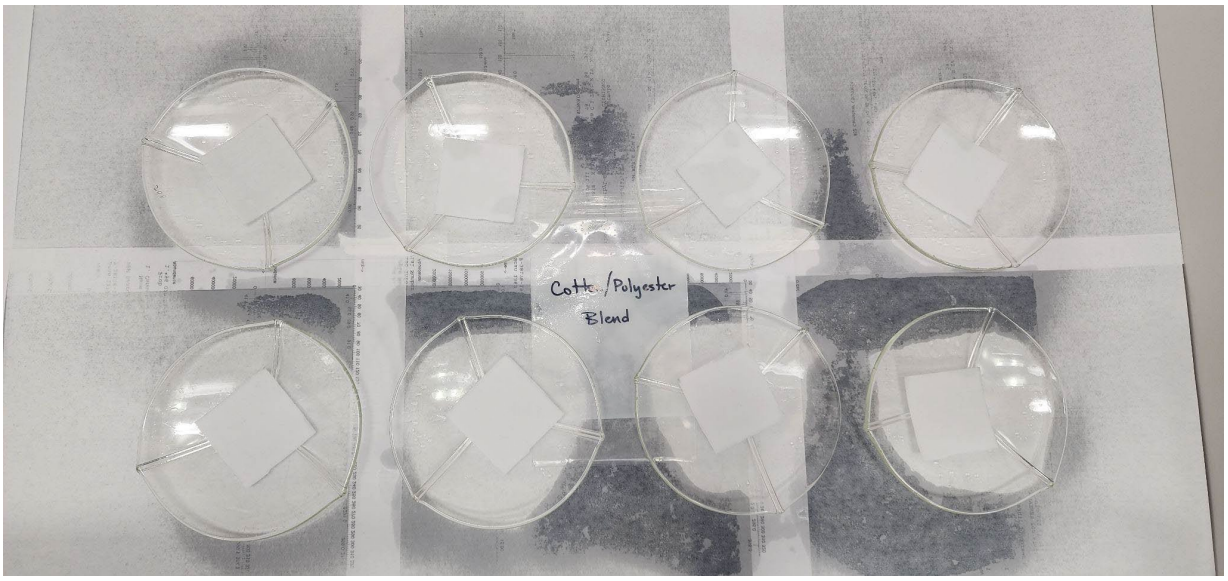

Fig. 2.2.1 Cotton/Polyester samples spray coated with Scotchgard

Once coated on both sides, the samples were allowed to dry completely overnight. After drying, the samples were then coated in the same manner on the front and back sides an additional two times. These same steps were repeated for all nine (9) cotton/polyester blend samples.

Once all fabric samples were spray coated a total of three times, the weights of the samples were taken to ensure the amount of coating for the samples were about the same. The average weight of the applied coating on the 100% cotton fabric was 0.39 grams and the average weight of the coating on the cotton/polyester blend fabric was 0.38 grams.

Samples were then divided into sets of nine each containing one 100% Cotton sample, one Cotton/Polyester Blend sample, one 100% Cotton sample coated with Scotchgard, and one Cotton/Polyester sample coated with Scotchgard. These sets were then labeled "Sample Set: to UC Davis" or numbered 1-8 and placed into labeled resealable bags. The set labeled "Sample Set: to UC Davis" was sent to Dr. Gang Sun in the University of California, Davis Biological Agriculture and Engineering group to obtain the contact angle of the fabrics. The contact angles are a way to evaluate whether the surface of a fabric has a hydrophobic or hydrophilic characteristic.[22] If a contact angle is greater than 90°, the surface is considered hydrophobic. A surface with a contact angle lower than 90° is hydrophilic.[23] The contact angles of the samples were observed and obtained by Sasha Eckstein and can be seen in Figure 2.2.3 and Table 2.2.

[22] T. M. G. Selva, J. S. G. Selva, and R. B. Prata, "Sensing Materials: Diamon-Based Materials." Encyclopedia of Sensors and Biosensors, 1 (2013): 45-72.
[23] Susanna Lauren, "7 Ways to Measure Contact Angle." *Biolin Scientific*, 25 May 2021, www.biolinscientific.com/blog/7-ways-to-measure-contact-angle.

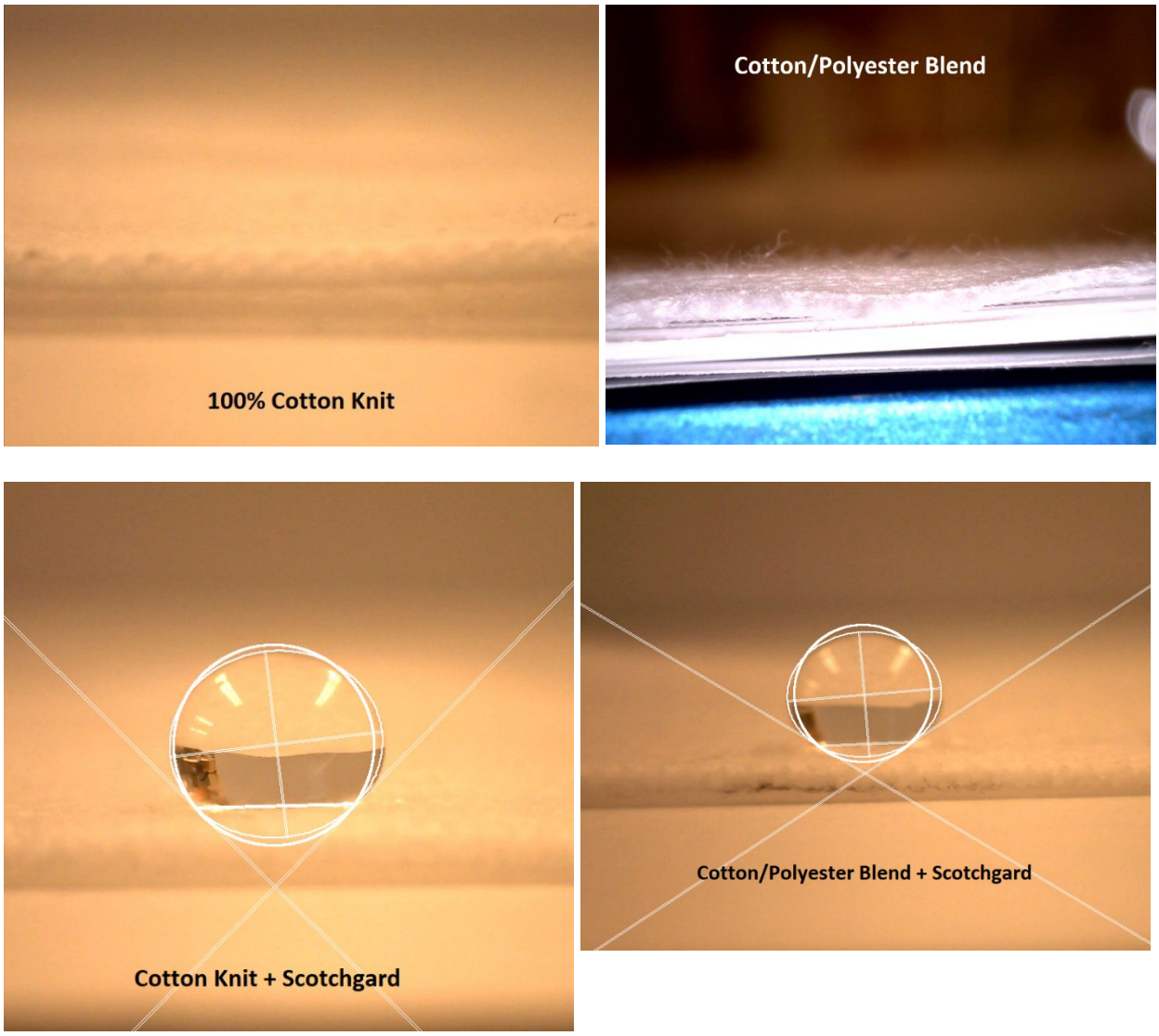

Figure 2.2.3 Photos used to find contact angles for 100% Cotton knit sample, Cotton/Polyester Blend sample, 100% Cotton knit with Scotchgard coating sample, and Cotton/Polyester Blend with Scotchgard coating sample.

As seen in Figure 2.2.3, the water droplets used to determine the contact angle for the samples coated with Scotchguard are visually repelling the water droplets and display contact angles above 90°, whereas the uncoated samples absorbed the water droplets, giving contact angles less than 90°. All fabric samples were ready for the blood application.

Table 2.2 Contact angles of samples

Fabric Sample Type	Contact Angle
Cotton Knit	0°
Cotton/Polyester Blend	0°
Cotton Knit + Scotchgard	178.83°
Cotton/Polyester Blend + Scotchgard	177.91°

Part III: The Blood

Completing one set at a time, the fabrics squares were individually placed on a watch glass. A one (1) milliliter (mL) plastic dropper containing one (1) mL of synthetic blood was positioned about six (6) inches above the sample. The dropper was emptied onto the samples and the fabric samples were left to absorb the blood for about one hour. After an hour, a pair of tweezers were used to pick up the fabric samples to let any excess blood slide off. The samples were then left to dry completely overnight. The resulting bloodied samples can be seen in Figure 2.3.1.

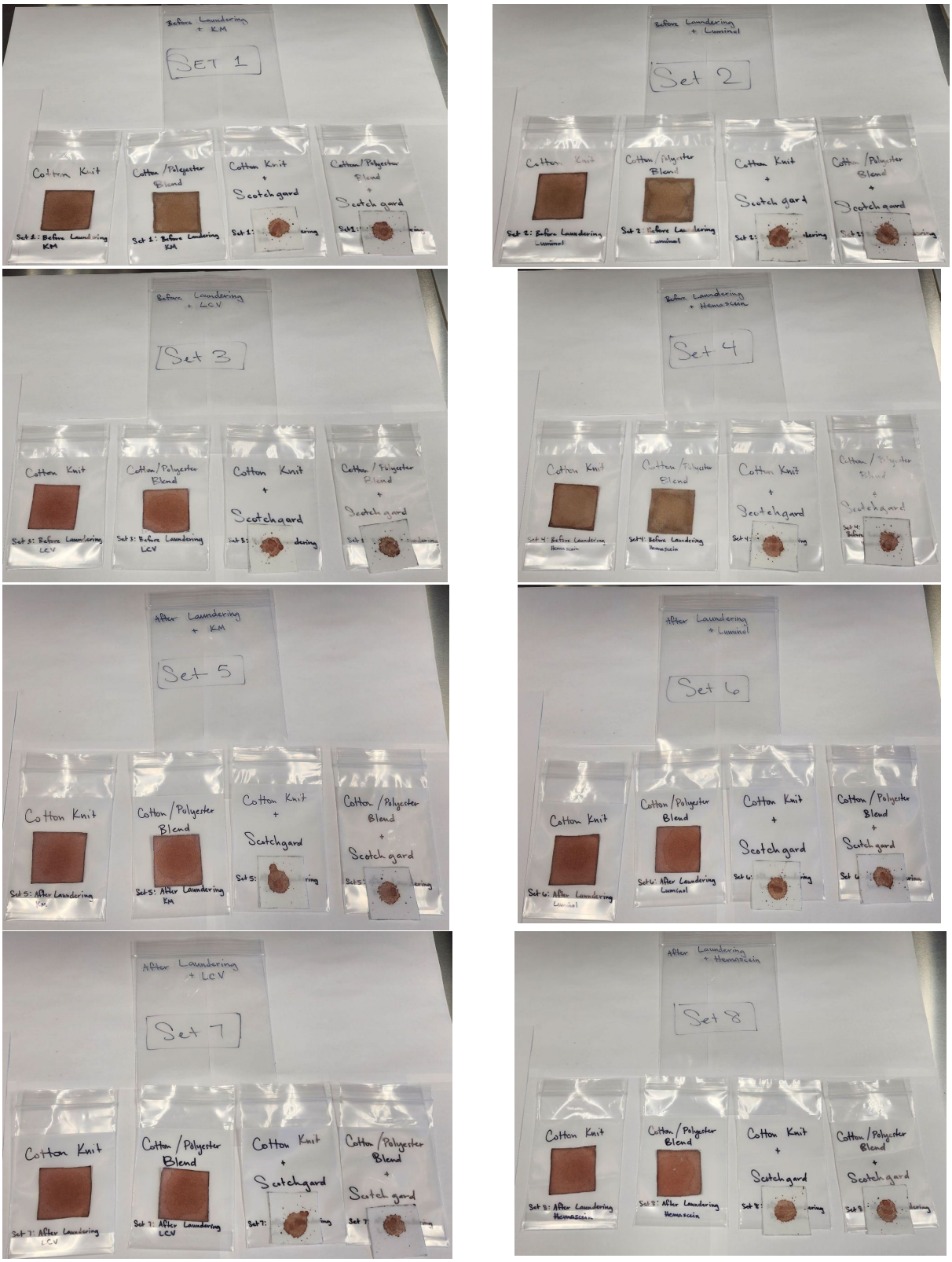

Fig. 2.3.1 Sets 1-8 after the application of Scotchgard (if applicable) and blood.

Part IV: The Launder

The researcher considered various factors to determine the most acceptable methods to test the fabric samples. Thus, the American Association of Textile Chemists and Colorists (AATCC) test methods were considered for the best inspiration to mimic industry standards of testing. Drawing from AATCC TM/135-2004, the following procedure was adapted and created to launder the samples. An Ethedeal tergotometer and a beaker were chosen to mimic the cycle of a washing machine. The laundering setup is depicted in Fig. 2.4.1. Kirkland's Signature Ultra Clean HE Liquid detergent was chosen due to it being the best value laundry detergent brand as stated by Consumer Reports.[24]

Figure 2.4.1 Mimicking a washing machine cycle using a tergotometer and beaker with water and detergent

To launder the samples appropriately, each fabric square sample was placed one at a time into a beaker that contained 200 mL water and 5 drops of the detergent. The tergotometer was lowered into the beaker and let to run at 640 rpm for 12 minutes. After each sample finished running the 12-minute cycle, they were taken out and placed on a

[24] Keith Flamer, "Best and Worst Laundry Detergents." Consumer Reports, 21 Jun. 2023, www.consumerreports.org/appliances/laundry-detergents/best-and-worst-laundry-detergents-from-consumer-reports-tests-a9342715268/.

clothesline to hang dry, making sure that the clothespins were secured at two corners of the square, as seen in Fig. 2.4.2. The samples were left to hang dry until completely dry.

As seen in Figure 2.4.2, most of the blood on these samples seemed to leave little to no visible residue on the fabrics after laundering.

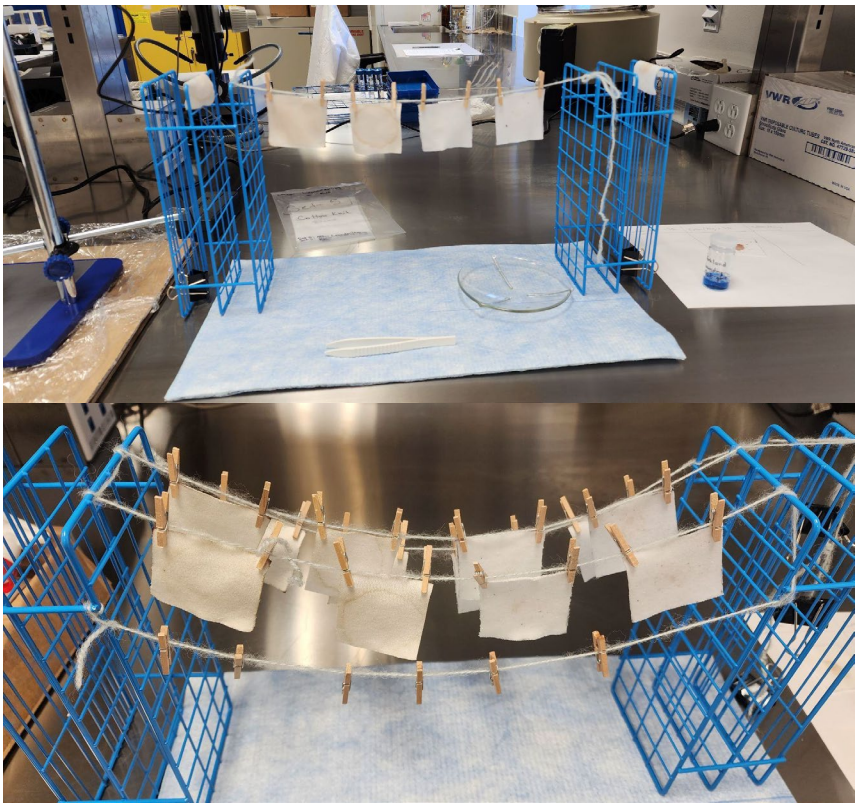

Figure 2.4.2 Laundered samples were hung to dry overnight or until completely dry on a makeshift clothes hanger using mini clothespins, test tube racks, and yarn. Top photo shows Set 5, whereas Sets 6-8 are shown in the bottom photo (front to back).

Part V: The Tests

A. Kastle-Meyer Test

Sets 1 and 5 were used for the Kastle-Meyer (KM) Test. The test was carried out as instructed by the reagent purchased from Carolina Biological Supply Company, shown in Figure 2.A. A cotton swab was moisten with distilled water. It was then used to swab the area of the fabric

with dried blood. One to two drops of 70% ethanol were added to the swab, followed by one to two drops of the Kastle-Meyer reagent. After the reagent was applied, one to two drops of store-bought 3% hydrogen peroxide was applied. Photos of the results on the swabs were taken and can be seen in Figure 3.A.3 and Figure 3.A.4.

Figure 2.A Kastle-Meyer Test set-up. The reagent and procedure for completing the test is seen in the top photo listing the components needed to complete the test from left to right: distilled water, 70% ethanol, Kastle-Meyer Reagent from the Carolina Biological Supply Company, and 3% hydrogen peroxide. The bottom photo shows Sets 1 and 5 that will undergo the KM testing procedure and the necessary cotton swabs (two in a pack) that will be used for each sample.

B. Luminol Test

For the Luminol Test purchased from Sirchie, sets 2 and 6 were placed in a dark room. The solution needed to execute the luminol test was created using the instructions provided by the kit purchased from Sirchie right before use. To create the luminol solution, the contents in pouch "A" were poured into the bottle labeled "B" and the supplied spray head attachment was screwed onto bottle "B" to shake thoroughly. With the sample sets laid out, the mixed solution was misted above the area of the sample fabrics and the light was shut off. The results of the test were then captured and can be seen in Figure 3.B.3.

Fig. 2.5.B Luminol Reagent in spray bottle.

Fig. 2.5.C Leucocrystal Violet Reagent in a spray bottle

C. Leuco-Crystal Violet Test

For the LCV Test, sets 3 and 7 were placed in a hood in the Drug Chemistry Wet lab of the New Orleans Police Department Crime Lab. To prepare the test purchased from Arrowhead Forensics, the solution was made following the provided instructions in the kit. In the provided spray bottle, the contents of components "B" and "C" were poured in. The spray head attachment was fastened to the bottle and shaken vigorously for about two to three minutes. After being mixed, the solution was sprayed above the fabric samples and the results were recorded. Results are shown in Figure 3.C.2.

D. Hemascein Test

For the Hemascein Test, sets 4 and 8 were placed in a dark room. The test was prepared using the directions given in the Hemascein kit purchased from Abacus Diagnostics right before use. There were several solutions to make for this test, the first being the stock solution. To prepare the stock solution, five milliliters of water were added to the Hemascein powder vial and mixed vigorously using a vortex mixer. One milliliter was taken from this stock solution and added to one of the spray bottles provided in the kit. One hundred milliliters of distilled water were added to this spray bottle and capped to make the "working solution." In the second spray bottle supplied in the kit, one hundred milliliters of 3% hydrogen peroxide were added and capped. To test the fabrics, the working solution was lightly misted over the samples from about one to two feet above. This was followed promptly by misting the same area with the 3% hydrogen peroxide sprayer to lightly coat the area. The lights were turned off, and using an Alternative Light Source (ALS) (450nm and orange filter goggles), the results were recorded. The results, albeit difficult to capture, can best be seen in Figure 3.D.3.

Fig. 2.5.D (Left to Right) Hemascein stock solution alongside two mister bottles filled with Hemascein Working Solution and 3% Hydrogen Peroxide, respectively, with an alternative light source (ALS) kit.

Chapter 3: The Results and Discussion

The presumptive tests, Kastle-Meyer Test, Luminol, LCV, and Hemascein, were used to determine the possible presence of blood on water-repellent cotton and cotton/polyester blends. To see the difference the tests would take on the fabrics and synthetic blood, I used both positive and negative controls for each test.

A. Presumptive Test: Kastle-Meyer Test Results

For the Kastle-Meyer Test, the change from a clear, colorless solution to a bright pink color denotes a positive result, indicating the possible presence of blood.

Fig. 3.A.1: Kastle-Meyer Test with synthetic blood on swab showing a positive result.

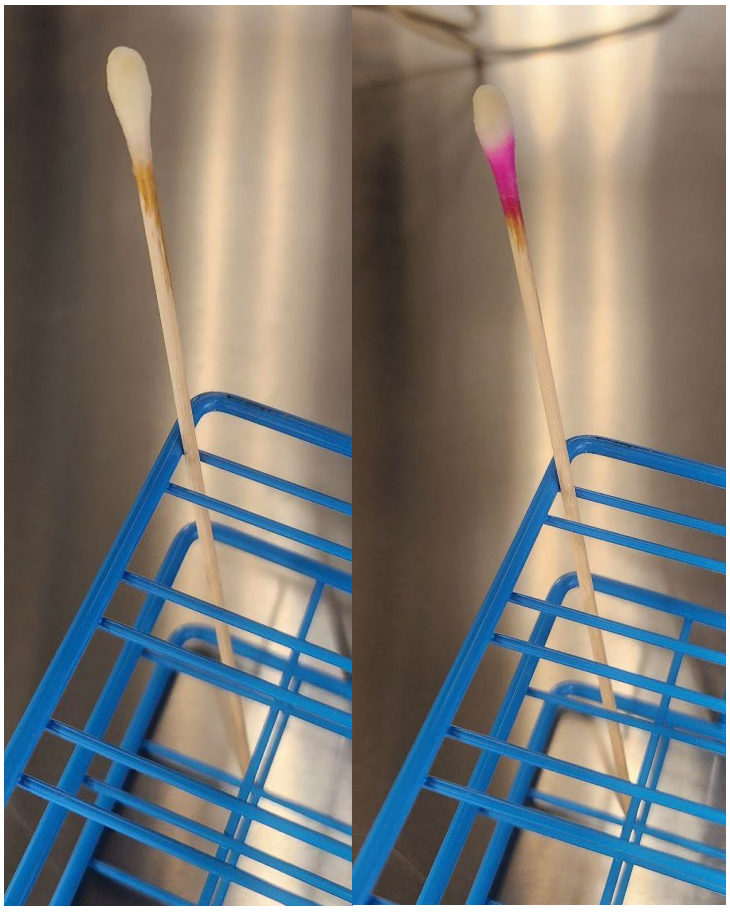

Fig. 3.A.2: Kastle-Meyer Test using a blank swab showing a negative result. Note that after some time, the adhesive used on the swab started to turn a bright pink color. This was taken into consideration when determining the results of the test.

In Figures 3.A.3 and 3.A.4, there are a total of twelve (12) swabs. This was a consequence of Set 5 being tested twice due to the uncertainty of the results. Some of the re-swabbed samples can be seen having a slight color change at the tips of the cotton swabs, but not as intensely as Set 1; therefore, these results were deemed inconclusive. As seen in Figure 3.A.2, the adhesive used on the swab started to turn a bright pink color after about three to five minutes. This was taken into consideration when determining the results for this test.

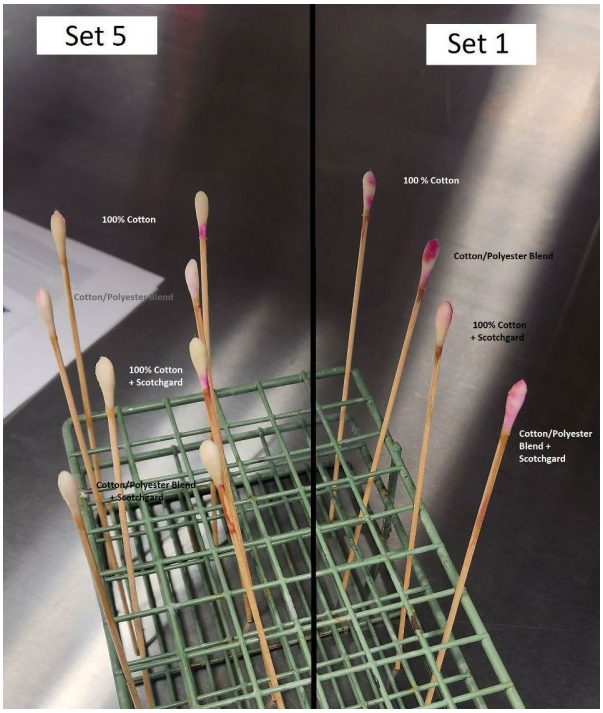

Fig. 3.A.3 Kastle-Meyer Test Results for Set 1 (right column) and Set 5 (left two columns).

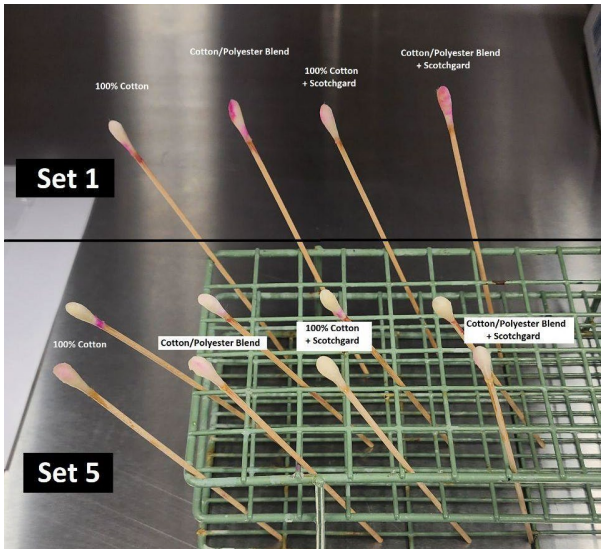

Fig. 3.A.4 Kastle-Meyer Test Results for Set 1 (top row) and Set 5 (bottom two rows) from a different angle.

B. *Presumptive Test: Luminol Test Results*

For Luminol, a blue chemiluminescence on the tested items in darkness indicates the possible presence of blood.

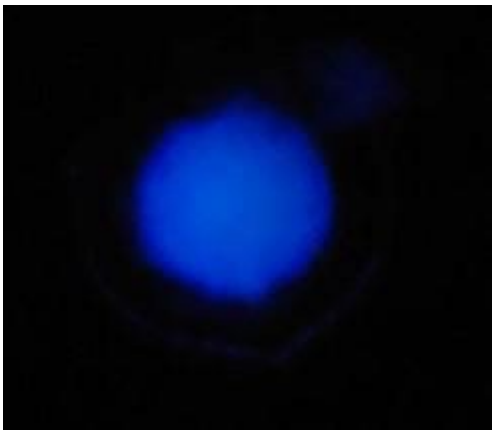

Fig. 3.B.1 Luminol Test with synthetic blood on wash glass showing a positive result.

Fig. 3.B.2 Luminol Test on fabric samples showing a negative result.

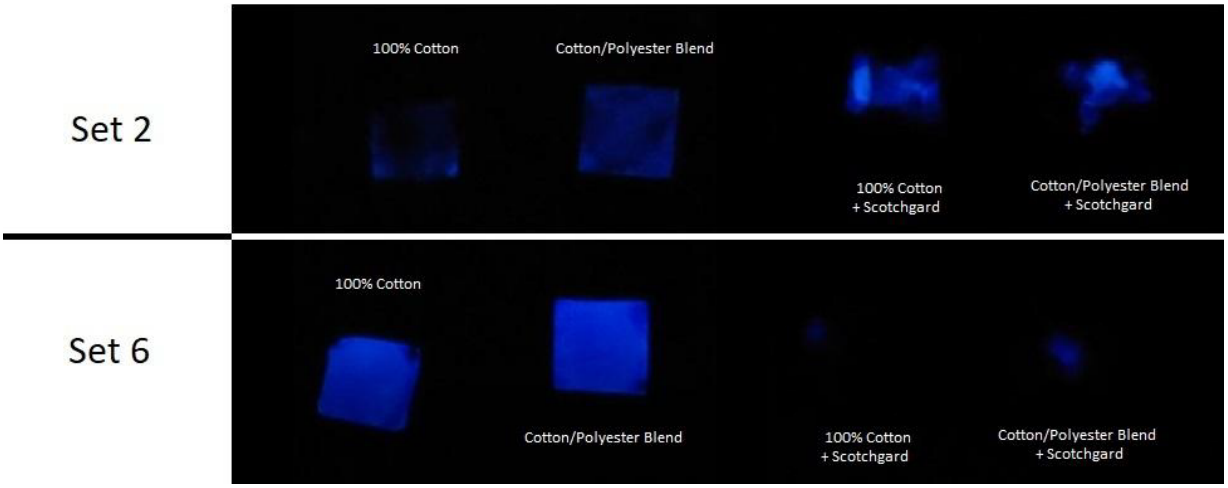

Fig. 3.B.3 Luminol Test on Set 2 (top row) and Set 6 (bottom row).

As seen in Figure 3.B.3., all the samples showed a chemiluminescence in the form of a blue color when the Luminol reagent was applied. It is important to note that about ten minutes had passed between applying the reagent and capturing the results. Because of this timelapse, the photos taken do not show the results of this test as vibrantly as when it was freshly applied. Although this test developed results quickly, the results also disappeared just as quickly, but the reagent could be applied repeatedly to show the results again.

C. *Presumptive Test: LCV Test Results*

For LCV, the change from a clear, colorless solution to a deep purple/violet color indicates a positive result for the possible presence of blood. Figure 3.C.1 shows a negative result on the fabric samples as well as positive results on drops of blood dotted on a watch glass.

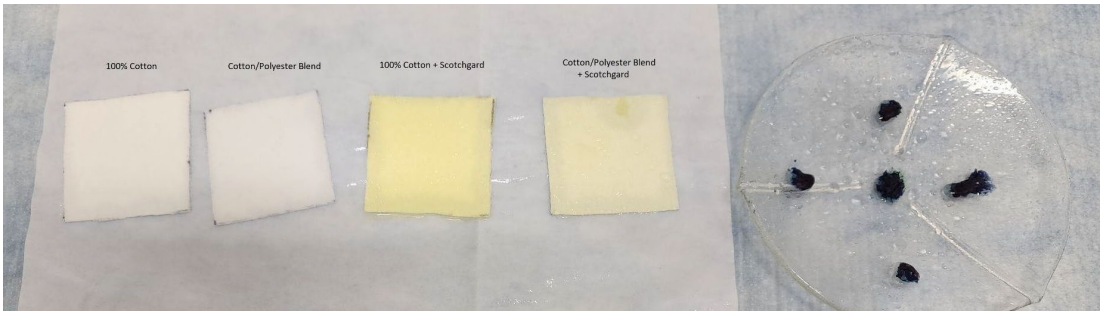

Fig. 3.C.1 Leucocrystal Violet Test used on fabric samples with and without the water-repellent coating showing a negative result (left) and on synthetic blood dotted on a watch glass showing a positive result (right).

For this experiment, Sets 3 and 7 were used to test LCV and the results can be seen in Fig 3.C.2. LCV was shown to detect the presence of blood for the unlaundered fabrics in Set 3 on both coated and uncoated fabrics, but showed inconclusive or negative results for the laundered coated and uncoated fabrics in Set 7. A closer look at the uncoated cotton and cotton/polyester blend fabrics in Set 7 shows some background staining. This type of background staining has been also seen in previous research done by Farrugia et. al. (2011) and could be from a possible reaction to residual blood.[25] For this reason, the results from the uncoated cotton and cotton/polyester blend fabrics in Set 7 were considered inconclusive.

Yellow staining on all the coated fabrics used in this LCV test was seen in both tested sets (figure 3.C.2) as well as the controlled set (figure 3.C.1) and was therefore not considered

[25] Farrugia et. al., "Chemical enhancement of footwear."

important in determining the results. This yellow staining is most likely a result of the LCV reagent reacting to the chemicals in the Scotchgard.

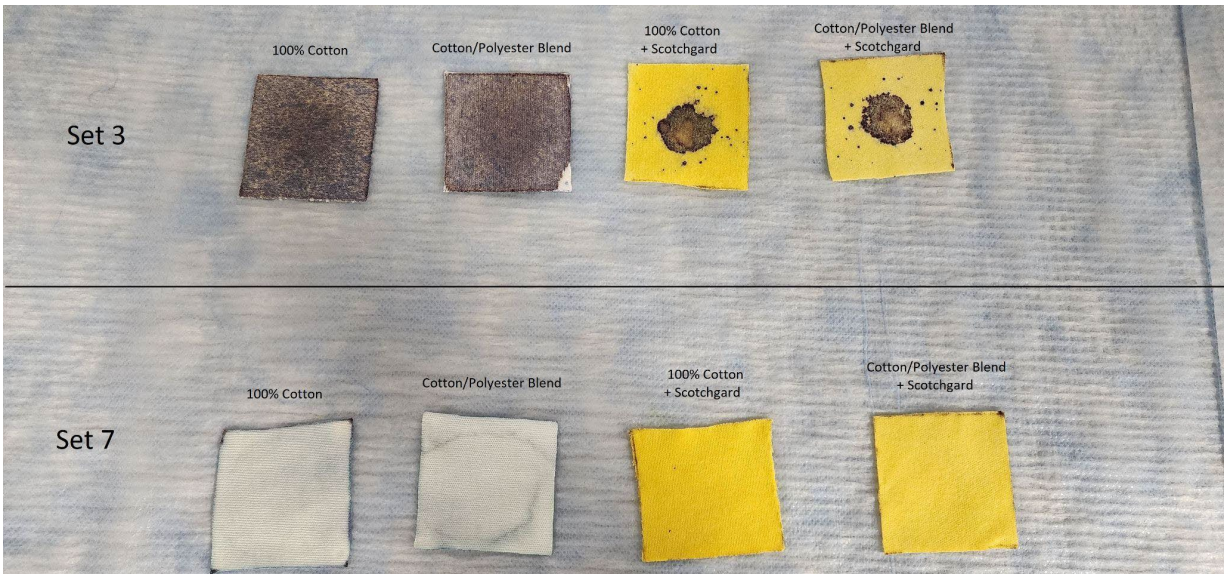

Fig. 3.C.2 Leucocrystal Violet Test results used on Set 3 (top row) and Set 7 (bottom row).

D. *Presumptive Test: Hemascein Test Results*

Similarly to Luminol, Hemascein shows a chemiluminescence in the possible presence of blood on the items in darkness with the results being only visible when using a red/orange filter. Instead of a blue chemiluminescence, Hemascein showed a green chemiluminescence glow. The results of this test were difficult to obtain on camera due to needing a red filter to see the chemiluminescence. I video recorded the results, but the photos captured from the video and seen in Figures 3.D.1 and 3.D.2 are regrettably not optimal.

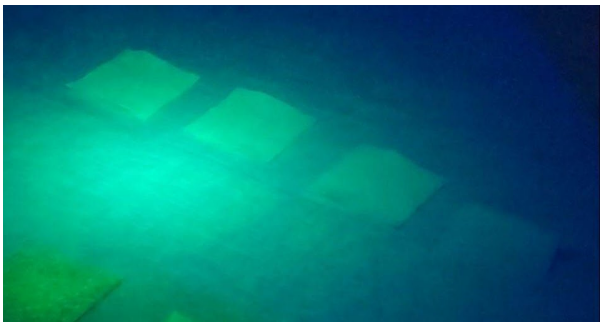

Fig. 3.D.1 Hemascein Test used on fabric samples with (right) and without (right) the water-repellent coating, showing a negative result using a red filter.

The controlled group of fabrics exhibited no chemiluminescence (Figure 3.D.1), whereas the controlled group of blood on a watch glass (not pictured) showed a green chemiluminescence around the edges of the blood pool. Sets 4 and 8 were used for the Hemascein test and all samples exhibited green chemiluminescence after about three to five minutes and after about two to three applications of the reagent and hydrogen peroxide.

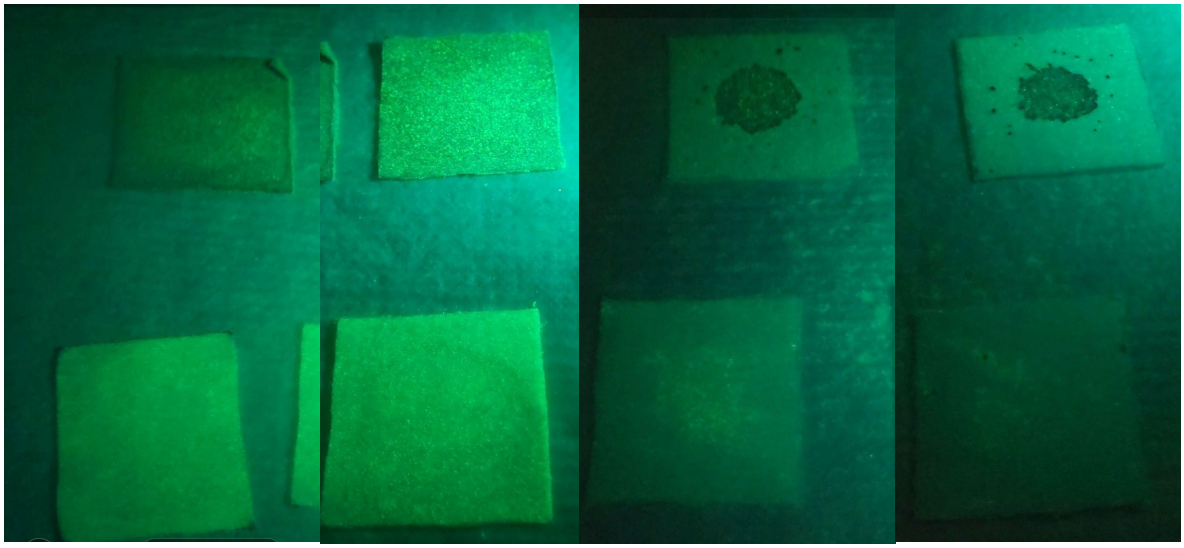

Fig 3.D.2 Hemascein Test results used on Set 4 (top row) and Set 8 (bottom Row).

Although the photos of the results taken and shown in Figures 3.D.1 and 3.D.2 do not do the in-person results justice due to having a red filter held up to the camera, the results witnessed by the author and my in-lab helpers are true to the results listed in Tables 4.1 and 4.2.

Hemascein took about three to five minutes to develop results, and the application of the test had more of a learning curve than the other tests used in this experiment.

Chapter 4: Conclusion

Overall, the Luminol and Hemascein presumptive tests worked the best across all samples in this experiment. All presumptive tests tested well on the unlaundered coated and uncoated fabrics (Table 4.1), but Luminol and Hemascein were the only two that worked well on the laundered coated and uncoated fabrics (Table 4.2). KM and LCV gave inconclusive and negative results for the laundered fabrics, with LCV exhibiting some background staining for the uncoated fabrics that were treated with blood prior to reagent application. The results did not show that water-repellent coating on fabrics significantly inhibited these tests in detecting trace residue of blood. The laundering employed in the study did not affect the detection ability of the testing agents as well.

Table 4.1 Before Laundering Results
"+" = positive result; "-" = negative result; "INC" = inconclusive result

	Type of Fabrics			
Type of Test	**100% Cotton**	**Cotton/Polyester Blend**	**Cotton + Scotchgard**	**Cotton/Polyester Blend + Scotchgard**
KM	+	+	+	+
Luminol	+	+	+	+
LVC	+	+	+	+
Hemascein	+	+	+	+

Table 4.2 After Laundering
"+" = positive result; "-" = negative result; "INC" = inconclusive result

	Type of Fabrics			
Type of Test	**100% Cotton**	**Cotton/Polyester Blend**	**Cotton + Scotchgard**	**Cotton/Polyester Blend + Scotchgard**
KM	INC	INC	INC	INC
Luminol	+	+	+	+
LVC	INC	INC	-	-
Hemascein	+	+	+	+

Further testing should be conducted since there was a small cohort in this study and many variables, such as temperature of laundering water, detergent ingredients, samples used, and reactions to the coatings, were not considered. This experiment was simple, fairly inexpensive to conduct, and could easily be replicated in any laboratory. The four presumptive tests used in this study were relatively easy to use, did not require expensive equipment, and could be purchased easily.

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Lastly, I want to borrow some words from the one and only Snoop Dogg and add:

"I want to thank me for believing in me, I want to thank me
for doing all this hard work. I wanna thank me for having
no days off. [And] I wanna thank me for never quitting."

Cheers to the good days, treasure the little things, love with all your heart, and be kind.

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