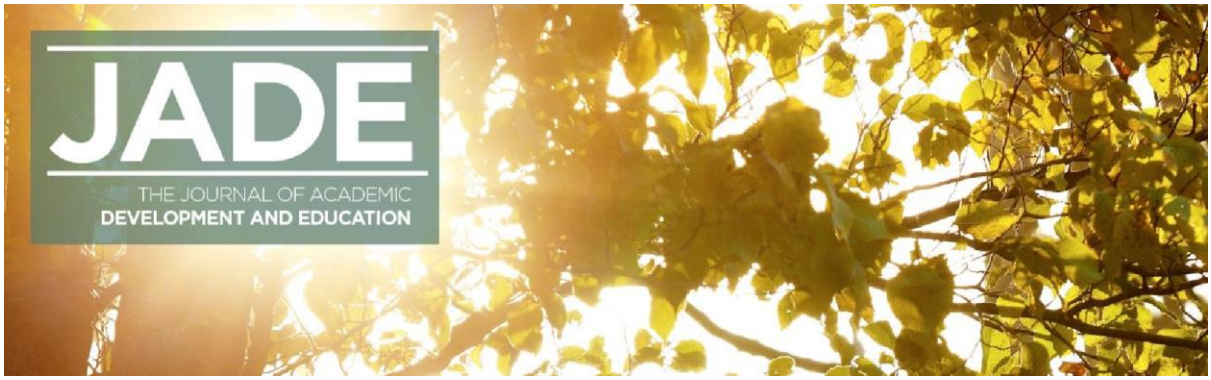


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**Article:**

# The Chemiluminescent imaging of blood on washed cotton and polyester fabrics

**Duwaraga Manoharan**

## Abstract

Luminol is a sensitive test used by forensic investigators to detect blood over a large area in crime scenes. When in contact with blood, luminol emits a bright blue light. This reaction, known as chemiluminescence, was utilised in this study to test luminol sensitivity on 60, 100% cotton and 60, 100% polyester swatches measuring 10cm by 10cm. All samples were washed once, twice or thrice with non-biological or biological laundry capsules, air-dried and subsequently sprayed with luminol and photographed in a dark room. Blood was detected on all 60 blood-stained swatches regardless of washing patterns or laundry capsules. The 60 unstained controls produced little or no chemiluminescence thus making luminol a sensitive and useful test for detecting blood on fabrics found in crime scenes.

## Keywords

Luminol, cotton swatch, polyester swatch, blood

## Introduction

Blood is common form of evidence found in crime scenes (Tobe, Watson and Daéid, 2007) and is easy to visualise under the naked eye due to its distinct red colour that indicates the presence of the red pigment haemoglobin (Mader and Windelspecht, 2016). This red colour provides a contrast between the blood and the background, but on dark or washed surfaces, it can be difficult to see (Butler, Chaseling and Wright, 2019). This important biological fluid is composed of two components - plasma and formed cellular components. These formed cellular components consist of erythrocytes (red blood cells), leukocytes (white blood cells) and platelets. Platelets are suspended in plasma and carry out important processes such as defence against foreign pathogens, transport of oxygen and carbon dioxide in the body and blood clotting (Bell, 2019). The discovery of blood in crime scenes has huge importance as leukocytes contain vital DNA sources in their nuclei (Bell, 2019) that provide important clues about the victims and suspects involved in the crime scenes.

However, if bloodstains have been diluted by planned and meticulous washing, detection can be limited. In this case, a solution called luminol is used to find blood. Luminol utilises haemoglobin in the blood to indicate the presence of blood in a crime scene (Bell, 2019). It is sprayed over a large area, in a darkened room and forms a blue colour to indicate that blood is present. It is also known as 3-Aminophthalhydrazide and 5-amino-1,4-dihydroxyphthalazine and 5-amino-2,3-dihydro-1,4-phthalazinedione in the literature (Huntress, Stanley and Parker, 1934), and is considered to be one of the most sensitive blood detection techniques (with sensitivities of one part per million (Shimamoto, DeFrance and Adair, 2013)) that is available for use in forensic investigations (Cassidy et al., 2017).

Luminol was first reported in 1902 (Huntress, Stanley, and Parker, 1934) and has been used by forensic investigators since 1937 (Adair and Shaw, 2005). Luminol exists as a crystalline solid that is dissolved in sodium hydroxide to make a stock solution and mixed with hydrogen peroxide and distilled water to make a working solution. The hydrogen peroxide acts as an oxidising agent and when in contact with blood, the ferric haem groups (the iron part of haemoglobin) decompose the hydrogen peroxide and act as catalyst for the oxidation of luminol (Cassidy et al., 2017). When this occurs, luminol releases energy in the form of bright blue light (Bell, 2019) and this forms a chemical reaction known as chemiluminescence. This chemiluminescence is a result of an excited molecule that is produced due to the oxidation of luminol. The surplus energy in the molecule is released as a light photon in the visible region of the electromagnetic spectrum (Bell, 2019). Once the area of interest is found, swabs of the luminescent area are taken and tested with confirmatory tests such as Kastle Meyer or leucomalachite green to establish the presence of blood (Brenzini and Pathak, 2018).

However, luminol is prone to false positives whereby it can be catalysed by something other than blood and cannot differentiate blood between different species. For example, household and industrial chemicals such as bleach contain hypochlorite ions that catalyse the reaction thus causing a false positive

(Quickenden and Cooper, 2001). As such, further analysis with tests such as RSID™ (Rapid Stain Identification-Blood), are used to establish that it is in fact blood that is present in the crime scene. This test, the RSID™, is dependent on the existence of a glycoprotein (a form of protein) called glycophorin A that transverses the membrane of erythrocytes (red blood cells) (Howard, Chaseling and Wright, 2019).

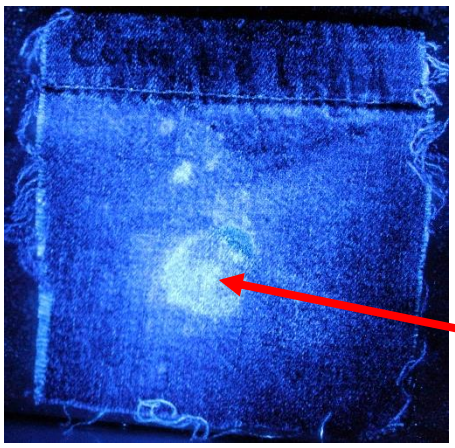


Figure 1 - reaction between luminol and blood (own image) on a denim swatch.

Blood was added to the middle of the swatch

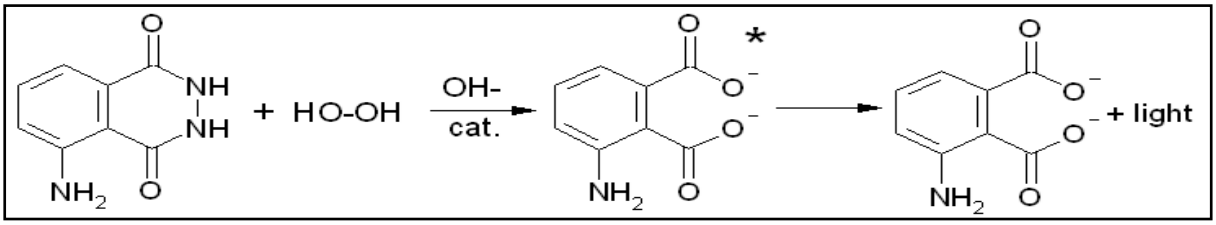



Figure 2 - the chemical reaction between luminol the haem group in blood (Seery, 2009).

Figure 2 is a schematic that shows the chemical reaction that takes place when luminol comes in contact with blood. The left-hand side of the reaction shows luminol ($C_8H_7N_3O_2$) being activated by the oxidising agent hydrogen peroxide ($H_2O_2$). The hydrogen from the two NH groups in the phthalate ring is removed, the two nitrogen Blood was added to the middle of the swatch from the NH group leave the ring as nitrogen gas, and two oxygen molecules are added by the hydrogen peroxide to the areas previously occupied by the two NH groups (Brenzini and Pathak, 2018). This forms 3-Aminophthalate, the middle structure, an excited and unstable molecule that has two negative electrons due to an electron moving from a low energy level to a higher one. The final part of the reaction shows 3-Aminophthalate emitting a bright blue light due to the molecule returning back to its ground state. This bright blue light is known as chemiluminescence. In order to view this chemiluminescence, a fully blacked out room is required. An example of this reaction between blood and luminol can be seen in Figure 1, imaged as part of this project, where blood was added to a 100% cotton denim swatch.

## Experimental

### a) Aims

1) Firstly, this project investigated the sensitivity of luminol in detecting latent bloodstains on two different types of fabrics after being subjected to machine washing once, twice and three times.
2) Secondly, this project investigated the role of non-biological and biological laundry capsules on removing bloodstains.
3) Thirdly, the study aimed to obtain quantitative information on chemiluminescence using the number of pixels that gathered from pictures taken of the luminol reaction.

**b) Preliminary Testing**

Preliminary testing was done to ensure that variables such as spraying techniques were consistent. 24 samples of swatches, measuring 10cm by 10cm were gathered from one 100% cotton denim dress, one 100% top and one 100% polyester jacket procured from charity shops. Of these 24 swatches, 12 were cotton and the remaining 12 were polyester. 6 samples of each material acted as controls and the remaining 6 samples had 0.3mL of ovine blood added to the middle. Using the available washing machines in the University Laundrette, the cotton samples were washed at 60 degrees and the polyester samples at 40 degrees as per the recommended settings from the clothing manufacturers. The samples were left to air dry with the temperature controlled to 21 degrees.

The luminol was made up using the Weber Method (Weber, 1966, pp.410–423). 3 different 500mL of stock solutions of luminol was made. These solutions contain hydrogen peroxide and sodium hydroxide and were further diluted with distilled water to make up a final volume of 500mL. The procedures for these solutions can be found below:

a. 0.354g of 3-aminophthalhydrazide will be combined with 62.5mL of 0.4 mol $dm^3$ sodium hydroxide. A final volume of 500mL will be made up with distilled water
b. 8 g of sodium hydroxide will be added to 500mL of distilled water
c. 10ml of 30% H2O2 will be mixed with 490ml of distilled water

Figure 3 - Blood stained and control Polyester Preliminary samples before they were washed (own image).

Figure 4 - Blood stained and control preliminary cotton samples before they were washed (own image).

## c. Materials and Methods

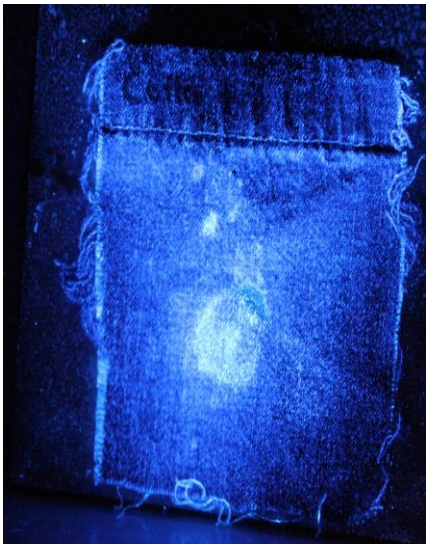
The experimental method was split into 4 test batches: the preliminary testing and 3 experimental conditions. In each testing condition, there were two controls and two testing conditions. A total number of 32 swatches were used in each testing section apart from in the preliminary condition where 24 swatches were used thus bringing the total number of swatches to 120. In each condition there were 16 polyester swatches and 16 cotton swatches. Blood was added to 8 cotton and polyester swatches, in a fume cupboard, and the remaining 8 swatches acted as controls. The swatches were then left to dry for 2 weeks.

Net washing bags were used to avoid the loss of the swatches in the machine drum. 12 swatches were split into 8 different laundry bags and after the first wash, 4 were taken out of each bag. After the second wash, 4 further swatches were taken out and the remaining 4 were the final wash. This was to adhere to the experimental conditions where the swatches in experiment 1 were washed once, twice for experiment 2 and thrice for experiment 3. This was the same for both the controls and the stained swatches. The controls and the swatches were not washed together so that no contamination of blood should occur. The clothes were then hung dry on an airer for 24 hours at a temperature 21 degrees. The washed samples were then stored in sandwich bags and photographed a week later.

## d. Results

The software programme COREL Photo Paint was used to provide exposure histograms for the swatches. Using the histograms and the blue channel, pixels from the 250 to 255 range were used. This is because most of the pixels appeared on the right-hand side of the histogram. The blue channel, along with the green and RGB (red, green, and blue) are three colour histograms known as the channel histograms. Each type illustrates the distribution of pixels in this channel (Pixel Magazine, 2017). The blue channel was used as it corresponds to the blue colour emitted due to the luminol reaction and as such was used to view the brightness level of the blue colour in the images. The idea of the percentage change was to show how much light has been received in the 250 to 255 range. The images were not cropped as it was hard to see the control samples where no visible outline was present.

Figure 5 - Cotton swatch washed with a biological laundry gel before luminol (on the left) and after luminol (on the right) with the histogram of the luminol reaction found below (own image).



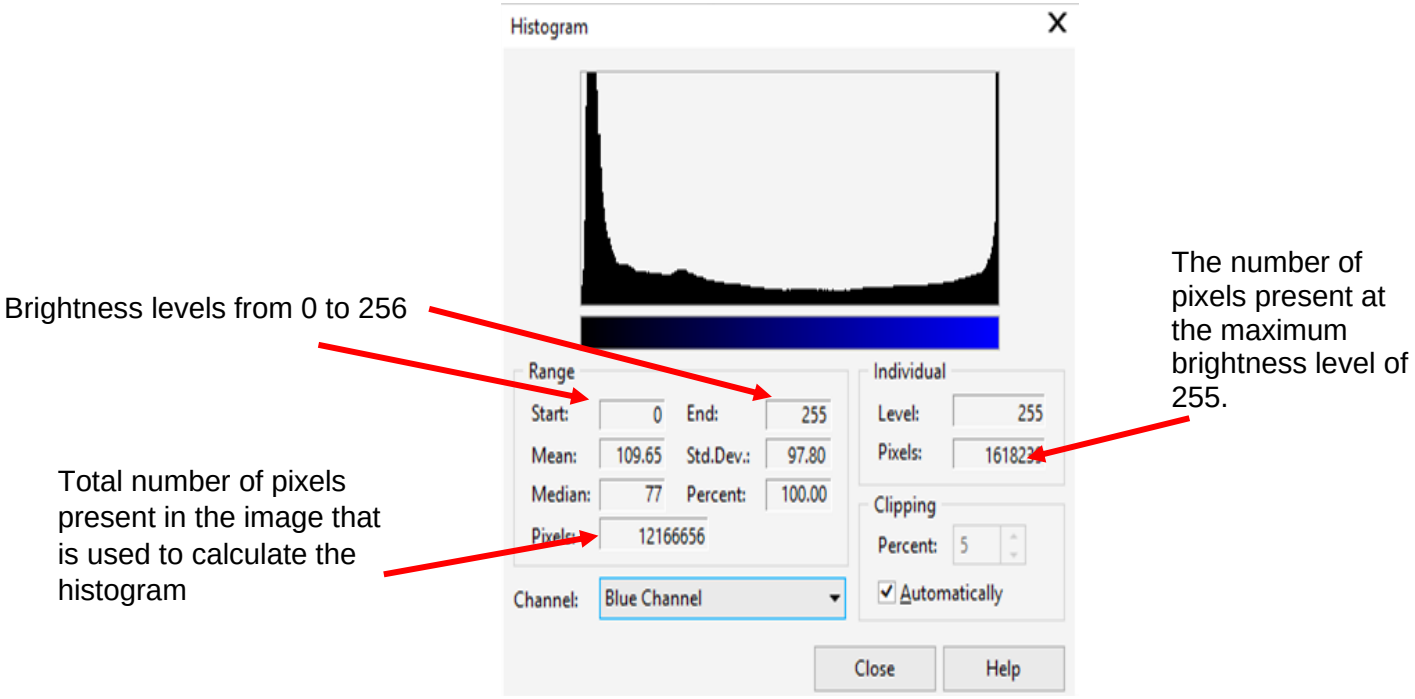



Figure 5 shows a cotton swatch, on the left, that has been washed once with a biological laundry tablet. Some blood can still be visualised in the centre of the swatch. The image on the right is of the same swatch but after luminol addition. The histogram of the chemiluminescent image is seen below the two images. The left-hand side of the histogram is showing the shadow detail and the right-hand side shows the highlights. The software also provides the mean, median, standard deviation, the total amount of pixels in the image, the pixels in a range and highlight clipping. The mean, median and the standard deviation are the measure of brightness in image. Clipping refers to the loss of detail when a certain part of the histogram is touching either side. This can be solved by altering exposure settings.

Chemiluminescence was detected on all the 60 blood-stained samples. The blood-stained polyester samples, throughout all conditions, had brighter chemiluminescence than the cotton swatches regardless of the detergent used. The corresponding pixels for the polyester swatch had the highest pixels in the preliminary condition; 32.2% for the swatch washed with non-biological laundry detergent and 29.1% for the biological detergent. Polyester swatches washed with the biological laundry detergent continued to have the highest pixel percentage in all conditions, but this reduced by the third condition. For example, the average pixel value in experiment 1 was 28.8%, 12.4% in experiment 2 and 7.2% in experiment 3. A similar pattern was also noticed in polyester swatches that were washed with the non-biological laundry detergent where the values of 27.2% in experiment 1, 8.5% for experiment 2 and 6.4% for experiment 3 were gathered.
In the preliminary condition, cotton swatches had values of 16.3% for the biological and 11.1% for the non-biological detergent. Much like the polyester samples, the pixel values decreased throughout the experiments as the washing number increased. For example, the cotton biological swatch had a value of 7.8% in experiment 1, 5.0% in experiment 2, and 5.2% in experiment 3. The non-biological detergent cotton swatches gave values of 11.1% in the preliminary, 11.4% in experiment 1, 5.3% in experiment 2 and 4.0% in experiment 3.

Cotton swatches washed with both the biological and non-biological capsules

showed discrepancies in the chemiluminescence intensity. For example, the cotton non-biological detergent had values of 3.9%, 15.5% and 15.0% in swatches 1, 2 and 3. This could also be seen in the swatches washed with biological capsule where values were 5.7%, 13.3% and 4.5% respectively. This discrepancy is a result of human error when handling the swatches during the drying and imaging period. Chemiluminescence was also detected in some of the control samples though these were less than 0.001% for most apart from the cotton controls for the biological detergent in the preliminary test where the value was 0.11%. Chemiluminescence was also noted on the tiles during the preliminary testing. Figure 6 is one such example where chemiluminescence was detected on both the background tile and the bloodied swatch. One of the tiles was then imaged on its own (Figure 7) and chemiluminescence was noted (pixel value of 0.2%) though not as significantly high as the swatches. The tile was not cleaned prior to imaging so it is likely that a catalyst was already present that produced a false negative with luminol. To combat this, tissues were placed on the tiles prior to spraying and imaging. The tissues were changed repeatedly to further prevent background chemiluminescence. Charts 1 to 4 in the appendix show the results for each condition more clearly.

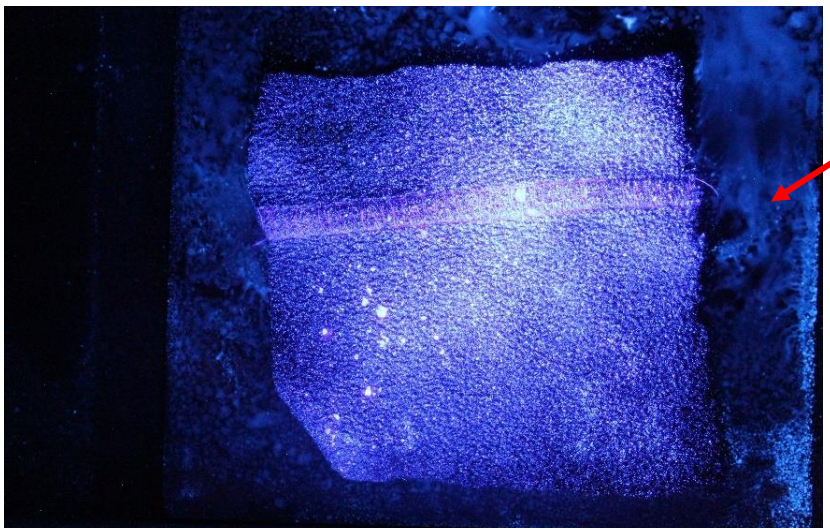



Figure 6 - Blood Polyester Swatch washed with biological laundry capsule

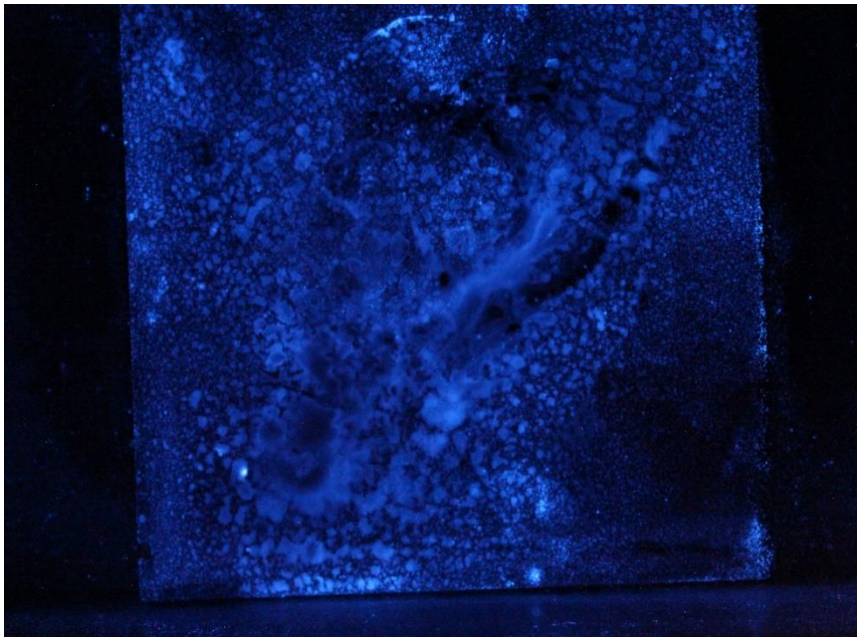


Figure 7 - Tile on its own showing chemiluminescence

The use of biological and non-biological laundry capsules was to see which performed the best at cleaning stains. Biological detergents contain enzymes to break down common clothing stains such as food and dirt (Persil, 2020). Non-biological detergents, on the other hand, do not contain enzymes (Persil, 2020). Both capsules had similar chemiluminescence intensity but the biological one worked better.

**Discussion**

Overall, chemiluminescence was detected on all 60 samples where blood was added. Luminol can react with even the smallest of traces so it was expected that it would detect blood from swatches that have been washed three times. The controls showed miniscule amounts of chemiluminescence when it should not have. Instead, it is likely that the luminol reacted with an unknown catalyst that was possibly present in the washing machines or the airer. The laundrettes are used daily by some 400 or so students who live in that accommodation and are only prewashed every so often. As a result, it is possible that dirt from the machines could have transmitted to the swatches. The best chemiluminescence detection was found in the polyester material

With regards to effective cleaning, experiment 1 and preliminary had the worst cleaning result as they were only washed once. This suggests that even at 60 degrees, some stains remained. Both the non-biological and biological laundry capsules can remove bloodstains, but biological capsules did a better job as the intensity across the preliminary and experimental conditions were less for these capsules. The use of pixels in this study helped with providing a quantitative analysis of chemiluminescent. Initial analysis of interpreting luminol emission involved providing qualitative statements using a verbal scale of 0 to 5 where 0 is no chemiluminescence and 5 being intense. However, this was abandoned in favour of software investigations. Studies by Tobe, Watson and Daéid (2007) and Cassidy et

al (2017), used statistical testing or computer software to analyse their results. Since DNA profile was not gathered in this project, statistical analyses were not undertaken, and instead the software package COREL Photo Paint was used. It provided useful information on the number of pixels available in the photographs in the form of exposure histograms. Better analysis would have involved cropping the pictures to show the reaction area but this wasn't fruitful due to low or no visibility of the samples in the controls.

**Future Research**

Further studies on blood detection in crime scenes should consider the use of visible reflectance hyperspectral imaging. This can reduce the use of luminol to detect blood as large quantities of luminol can be expensive and time consuming. For example, Edelman, van Leeuwen and Aalders (2015) used 4 different visible reflectance hyperspectral methods, in the range 400- 720nm to enhance and contrast bloodstains on 12 different fabrics. This method proved to be more successful than white light photography as blood was detected on all fabrics and are also convenient for crime scenes as the camera system is portable and wireless. Hyperspectral imaging can also be used to provide age estimation of blood stains (Edelman, van Leeuwen and Aalders, 2015) thus providing vital clues about the date of crime which luminol, currently, is unable to do so. Along with hyperspectral methods, infrared (IR) spectroscopy can also successfully be used to provide contrast between latent bloodstains and the background (Lin et al., 2007).

**Conclusion**

The aims of this project have been met successfully. The project showed that luminol is indeed a sensitive solution and can detected latent bloodstains on swatches that have been washed up to three times and using two different laundry detergents. The results of this project also indicated that elimination of blood in fabrics proved to be a difficult task as chemiluminescence was detected even after three washes. This suggests that the fibres used in the swatches are strong as it proved difficult to get rid of the blood even after multiple washes. The use of pixels gathered from photographic evidence is a novel way of quantifying chemiluminescence intensity and helps with understanding the sensitivity of luminol. Further studies should ponder upon the integration of pixels in quantifying chemiluminescence to improve up the reliability of this technique.

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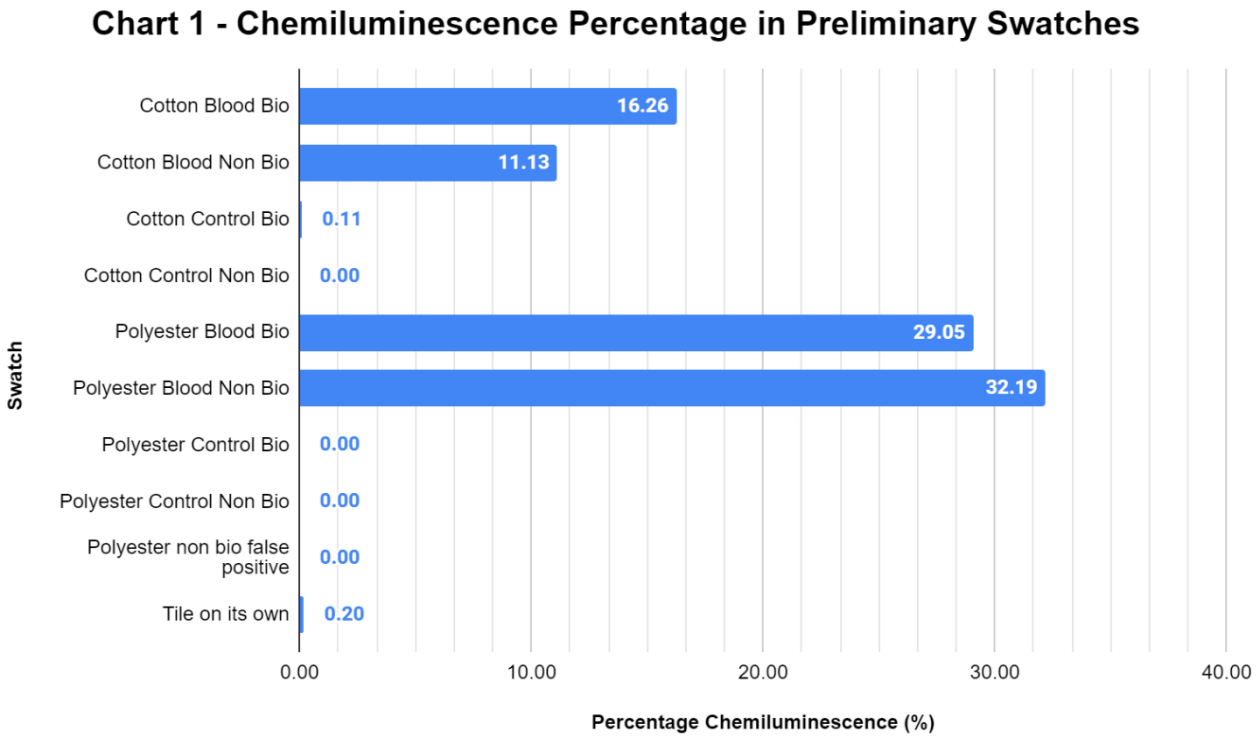
**Appendix**

Chart 1 – Chemiluminescence percentage in preliminary swatches



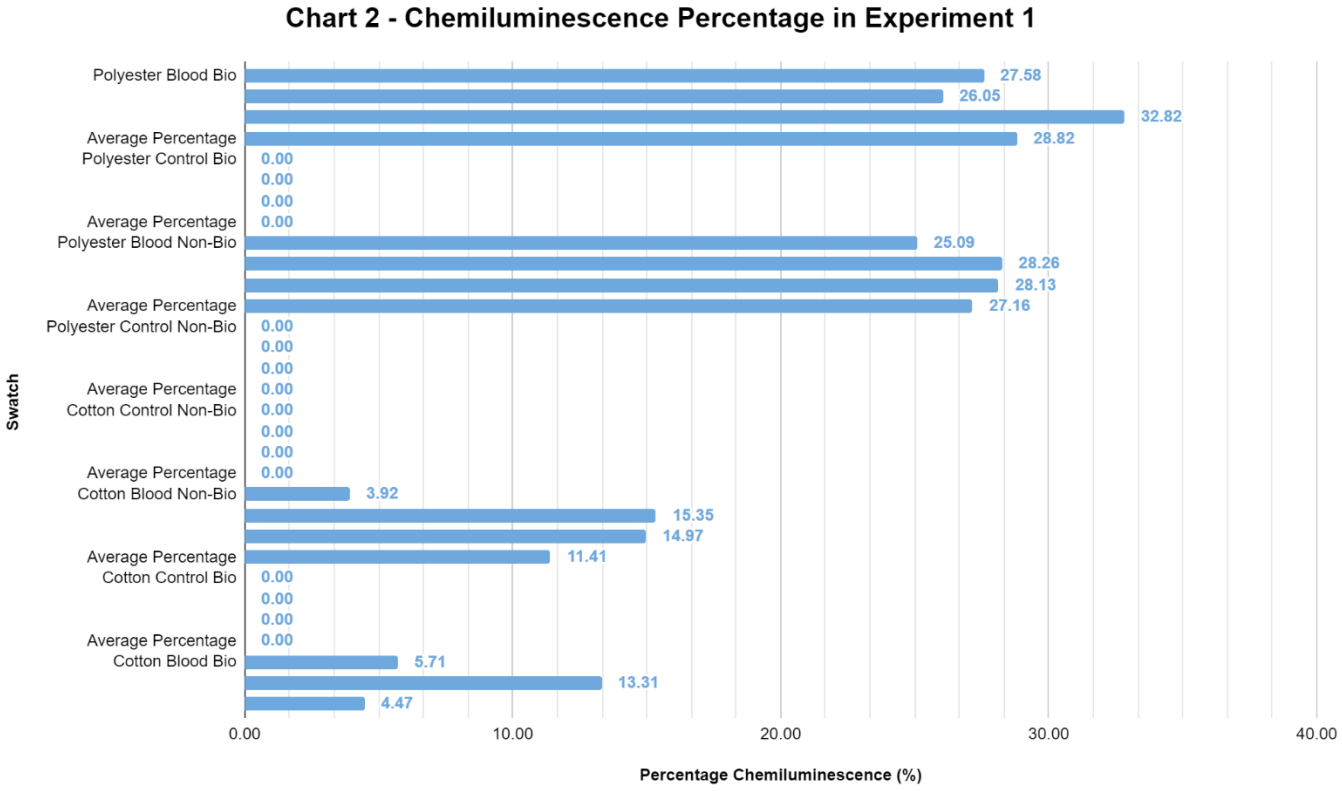
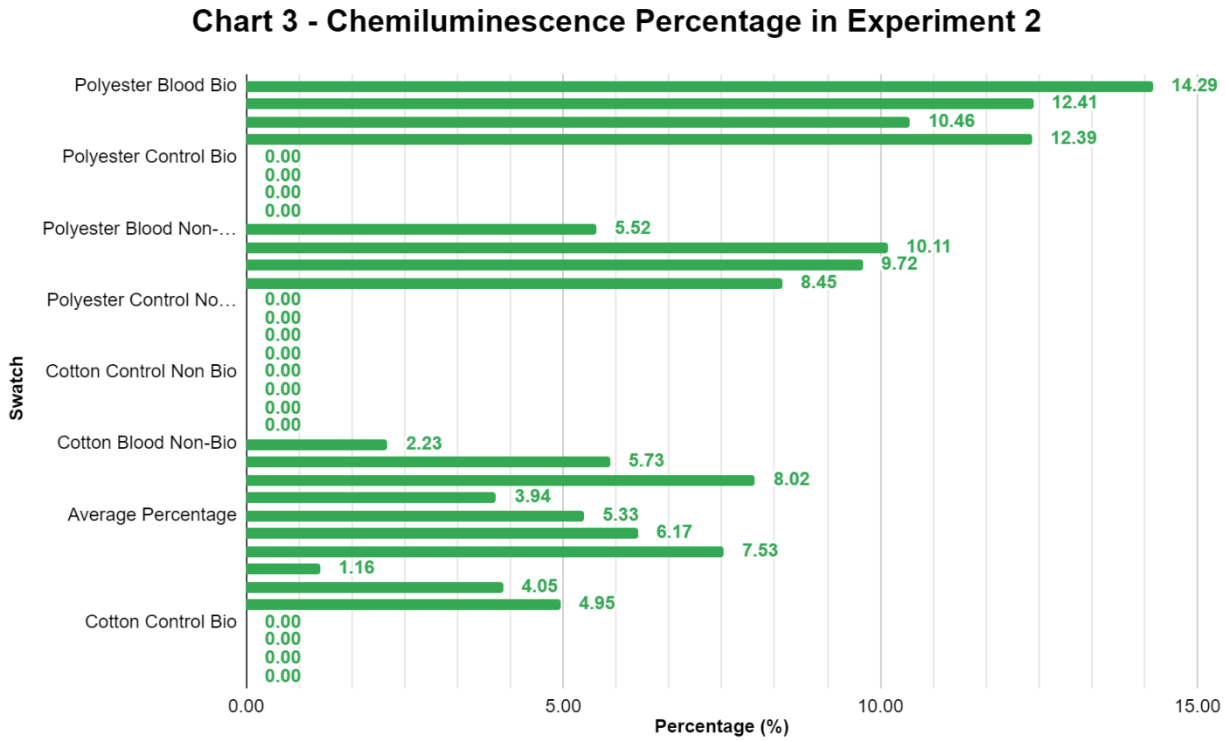




Chart 2 – Chemiluminescence percentage in Experiment 1

Chart 3 – Chemiluminescence percentage in Experiment 2



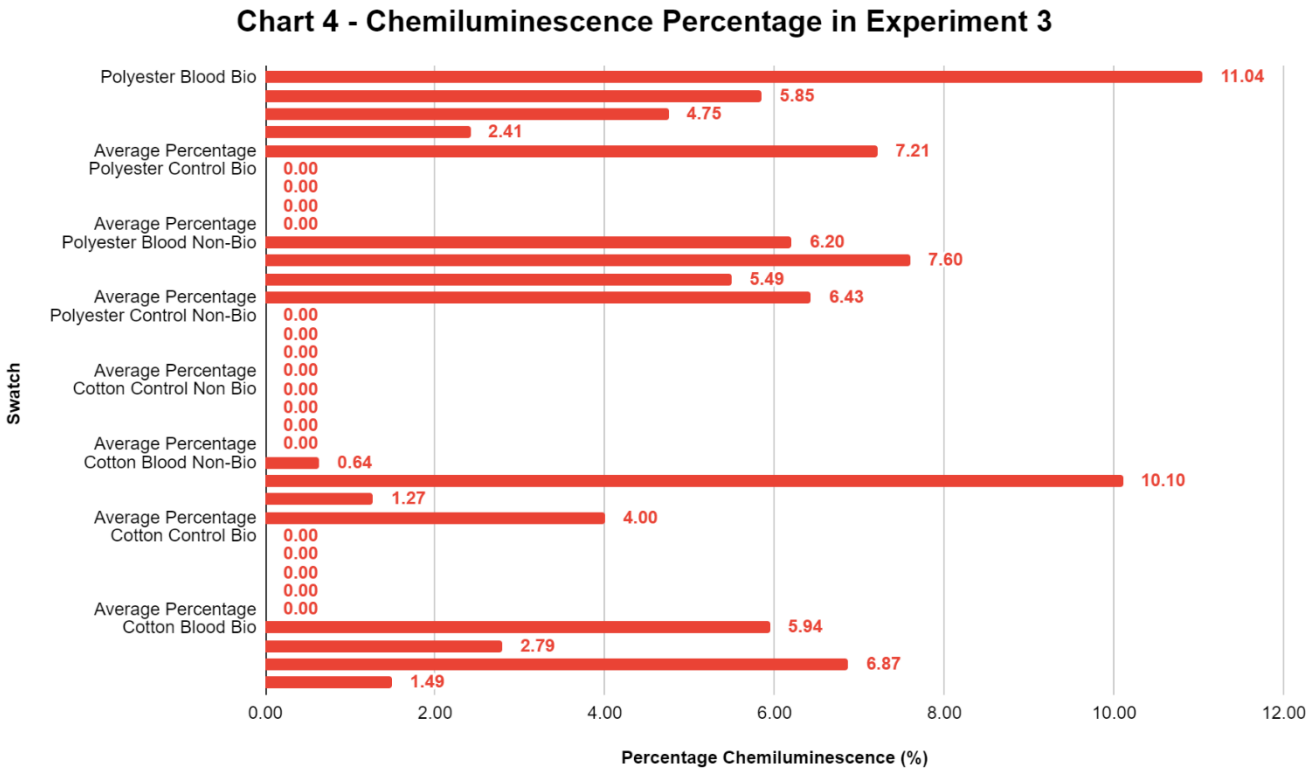




Chart 4 – Chemiluminescence percentage in Experiment 3