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A comparison study of the detection of bloodstains on painted and cleaned surfaces with luminol

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Highlights

- Bloodstains concealed with solvent based paint need a lower number of layers.
- Distinctive patterns in the surface of the tile when observed under the microscopy.
- Bleach tiles gave a general luminescence on the whole surface.
- More layers of paint were necessary where the bloodstains were not cleaned.

Abstract

There seems to be a limited amount of research about the detection of concealed bloodstains on painted surfaces. The bloodstains on walls and floors are often removed by cleaning, in some cases the surfaces are painted by the perpetrator after committing a violent crime in order to hide the crime that has occurred. The study hereafter extends and deepens on previous researches by investigating the detectability of horse bloodstains on painted ceramic tiles as a function of the number of layers of paint. In this study luminol was used as a reagent to detect the bloodstains. The study focuses on two types of paints: water based and solvent based paint. This study also investigates the effectiveness in reducing the detectability of bloodstains on ceramic tiles using four different cleaning methods pure water, soap with water, wet wipes, and bleach. In the experiment the bloodstains were cleaned at various intervals of time after the deposition (two minutes, fifteen minutes and one hour). The study concluded that the bloodstains concealed by layers of solvent based paint are less likely to be detected by luminol compared to water based paint. The study also concluded that the tiles cleaned with bleach are recognisable from the other ones cleaned using other methods. In each study the duration of the reaction was timed, highlighting the differences in the cleaning methods.

Keywords: Luminol, paint, bloodstains, cleaning methods, chemiluminescence, bleach.

1. Introduction

Different types of body fluids are found at a crime scenes, ~~and~~ the most commons ones are blood, semen and saliva, other body fluids like vaginal fluid, urine and sweat can also play an important role in the identification of DNA [1]. There are various presumptive and confirmatory tests that are used to identify and confirm the presence of body fluids [1]. Blood is composed of many components, the most abundant being red blood cells. The red blood cells transport oxygen around the body, and they do not have DNA. DNA is found in the white blood cells, their job is to repair the wounds and fight infections. The first defence of the body, when bleeding is to try and stop the blood lost by sending white blood cells to the wound to try and repair it. In doing so if a large or deep wound is inflicted many white cells will be sent out with the flow of blood. This results in a higher concentration of white blood cells in the immediate area where the first wound was inflicted [2]. To have a positive blood identification, it is required to have a presumptive test followed by a confirmatory test. There are various presumptive tests for blood, such as: *Benzidine*, *Tetramethylbenzidine (TMB), /Hemastix, Luminol*, *Fluorescein, leucomalachite green test (LMG)* [3]. When using leucomalachite green and Kastle Meyer tests, the samples are not tested directly, like in the case of luminol, but are tested indirectly. Indirectly testing is done by using swabs or filter papers, and spraying or dampening the swab in the solution. Luminol and Polilight are the methods most commonly used during crime scene examination [1]. These tests with the aid of bloodstain pattern analysis can provide an indication of the sequence of events at a crime scene, by showing an apparent amount of blood loss, the movements of the victim, the movement of the weapon used and the site of cleaning. Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione) is a reagent that produces a chemiluminescence when mixed with the smallest amount of oxidant. This phenomenon occurs when a molecule capable of fluorescing is raised to an excited level during the chemical reaction; when the electron goes back to the ground state, energy in the form of light is emitted [4]. Luminol is by far one of the most sensitive tests for blood, and produces a short-lived fluorescence [5]. After been identified with luminol, the routine procedure for blood is to swab the spot of luminescence and test it with Kastle Meyer or leucomalachite green tests [6]. Luminol is only considered as a presumptive test for blood because there are other substances capable of giving a positive reaction with luminol itself; these other substances may be bleach, plant peroxides and some metals, the reaction between luminol and these other substances are called false positives [7]. It is sometimes possible to distinguish the way luminol reacts with blood or with bleach. In the reaction with blood the luminescence is intense and can last for several minutes, while in the reaction with bleach it is analogue to a burning sparkler or twinkling stars [8]. Shaler [9] mentioned that flashes of light are an indication of a false positive reaction. It has been shown that primary and secondary amines inhibit oxidative chemiluminescence, tertiary amines actually increase the chemiluminescence [10]. Luminol works by catalysing the reactions within the iron group that is present in the haemoglobin. Luminol is transformed into a base that strips off the hydrogen from the two nitrogen's in the phthalate ring. Peroxide adds two oxygen molecules across the phthalate ring and the two nitrogen's in the ring leave as nitrogen gas. 3-Aminophthalate is then formed. This molecule is in the excited state in which it emits light when returning back to the ground state. Once the light has been emitted it is not possible to emit anymore light until more luminol is added to the area of interest. This type of reaction is not reversible since the molecule has expelled Nitrogen [4]. Luminol is also known to be able to detect blood from fresh stains to seventeen-year-old bloodstains and can also be used to detect blood under several coats of paint [6]. The research work undertaken by Bily and

Maldonado [8] for the detection of blood under several layers of paint suggested areas that can be further investigated. For example, the application of paint through a sprayer instead of using a roller, improvement in the photography and the ability of different types of paint to cover the surface stained with blood.

The aim of this work is to try to master the uncertainties of previous research work by trying to understand how well different concealment methods can eliminate detection of bloodstain traces at crime scenes.

2. Methodology

Eight ceramic tiles were used as a surface, four measuring 90x90 mm and other four measuring 150x150 mm.. The cleaning products used were tissue, wet wipes, hand soap, lemon bleach “Tesco”. Manila paper was used to cover all working surfaces to protect them from blood contamination. Defibrinated horse blood was used in place of human blood to stain the ceramic tiles. Layers of water-based paint and solvent-based paint were used to conceal the bloodstains on the ceramic tiles. The Kastle Meyer reagent, Hydrogen peroxide, Sodium hydroxide, and Luminol reagents were use to identify the presence of bloodstains under layers of paints. Canon SLR camera EOS 50D was used to capture the images and the luminescence.

2.1.Tile Preparation

Four tiles were washed with fresh water for each type of paint used. The methodology used was the same for both types of paint. For identification purposes, on the smooth side of the tile the time in which the bloodstain was cleaned and the type of paint used were recorded. A first coat of paint was applied on each tile on the rough side (the paints used were: water based paint Home of colour, Pure Brilliant White, Silk emulsion for walls and ceilings; and Crown, eggshell, pure Brilliant White, Solvent based for interior wood & metal). The paint was allowed to dry for twenty four hours. One tile for each type of paint was kept as a control. On the remaining tiles a drop of defibrinated horse blood was deposited with the use of a Pasteur Pipette. The control tile was kept separated from the bloodstained tiles to avoid any contamination.

2.2. Bloodstain cleaning, Kastle Meyer reagent and painting

The tiles were cleaned with three different cleaning methods on indicated times (see table 1). After each tile was cleaned, three random spots for each tile were swabbed and tested with the Kastle Meyer Reagent. A batch control of the swabs where also tested with the Kastle Meyer reagent. A first layer of paint was placed with a small brush; each layer of paint was left to dry for twenty four hours. Each tile was composed of four rows, row 1 no paint, row 2 one layer, row 3 two layers, row 4 three layers of paint.

Table 1. Cleaning methods and time past after cleaning

State of Blood stain when Cleaned	***Tile Number***	***Time before cleaning (min)***	***Types of cleaning per Tile***
Dry	1	60	Soap Water Wet Wipes No cleaning
Semi dry	2	15	Soap Water Wet Wipes No cleaning
Fresh	3	2	Soap Water Wet Wipes No cleaning

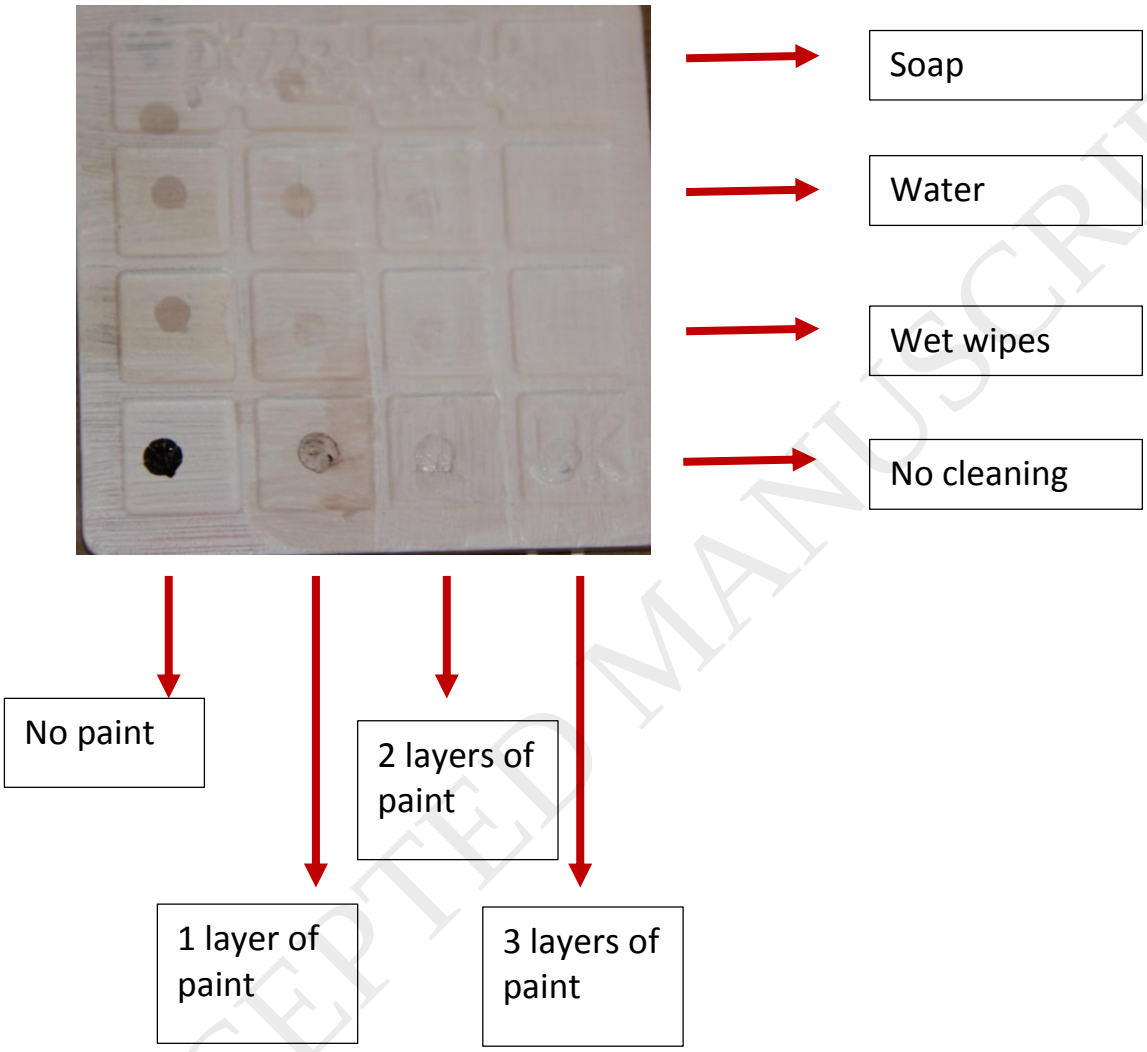

Figure 1. Shows the tile preparation and the cleaning methods.

2.3. Luminol Preparation and Test

The luminol was prepared following the indications and quantities on table 4. The luminol solution was kept separate in the three glass flasks and mixed in a glass spray bottle right before their use. 10 mL of each solution were mixed together and then shaken and left to rest for a few minutes before their use. The luminol test was performed in a room where it was possible to have a complete dark environment. A photograph for each tile reacting to the Luminol was taken with the aid of a Canon EOS 50D mounted on a tripod. The camera was set with the shutter speed of thirty seconds. First, the tiles with water based paint were tested, in the following order Control, Dry, Semidry and Fresh. Each tile was laid on a clean table with Manila paper; the camera was focused with the lights on. When the lights were turned off, the luminol was sprayed on the tiles. Each tile was sprayed approximately ten times. On conclusion of the luminol experiment on each tile, three more layers of paint were added following the procedure described above.

Table 2. Quantity of Luminol used

Solutions and Reagents	***Quantity***	***Final Concentration in Molarity/Normality***
Sodium Hydroxide	8g in 500mL of Water	0.4N
Hydrogen Peroxide	30% in 490 mL of Water 0.354g in 62.5mL Sodium hydroxide	0.176 M
Luminol	0.4M to final volume of 500mL in Water	0.004 M

2.4. Bleach Study

The methodology used in the first part of this experiment has already been explained above (follow section 2.1) this time by using two tiles for each type of paint. Once the tiles were prepared, one tile for each kind of paint was washed with lemon Tesco Bleach by applying a layer of bleach on the surface of the tile and letting it rest for three to five minutes, then the same tiles were rinsed vigorously under running water. The tiles were allowed to dry for fifteen minutes. The luminol was prepared while the tiles were drying. The dried tiles were tested with luminol following the luminol preparation table 4 in section 2.3 and luminol test. On the second set of tiles (where no bleach was applied), nine drops of blood were applied with the help of a pipette. Each row of three drops was cleaned with different timing (the same times used for the experiment above) but all of them were cleaned with bleach and tissue paper. After the tiles

were cleaned the luminol test was performed using the same procedure explained earlier (section 2.3). On conclusion of the luminol test, on the tiles, a layer of paint was applied, and the luminol test was repeated.

2.4. Microscopical study of the surface of the tiles painted with the two paint types in examination

The tiles used during the experiments were observed and compared under the high-power microscope on reflection mode, Zeiss Axioskop2 microscope was used with the magnification of 100X. A drop of defibrinated horse blood was deposited with a Pasteur pipette on the controls tiles of the bleach test.

3. Results and Discussion

When analysing the photos taken during the experiment, the author realised that it was difficult to estimate the amount of light emitted without a detector. So to keep coherence during the experimental work, a table with standards of amount of light was created using one of the photo taken during the experiment as reference and then applying the same reasoning to all the photos.

Table 3. Numerical value of amount of light observed in the luminol reactions

Amount of Light Observed	***Numerical Value***
+++	1.5
++	1.0
+	0.5
-	0

3.1. Kastle Meyer reagent on painted tiles

Two control samples were run, and both of the controls were negative. The Kastle Meyer positivity test is measured by a change of colour in the swab, turning pink if it is positive. The Kastle Meyer test is a presumptive test for the presence of blood, it is usually performed after the detection of blood with Luminol in order to confirm the presence of hidden or cleaned up blood on surfaces. The reason the Kastle Meyer test was performed before the luminol test was to observe if the use of painted tiles instead of wall sections would interfere with gathering of results. From the results on table 4 and 5 it was not possible to observe a pattern in the type of cleaning, but it was possible to affirm from the results that more positives results were obtained when the bloodstains were cleaned in the dry state. This might be because the longer the bloodstains were left, the more red blood cells were deposited on the tile. Therefore, providing more haemoglobin to react with the Kastle Meyer reagent. The two types of paint showed similar results.

- Water-based Paint

Table 4. Kastle Meyer reagent on water-based tiles after the washing of the Bloodstains

State of Bloodstains when cleaned	*Row swabbed*	*Type of cleaning*	*+/−*
Dry	1	No cleaning	+
	2	Water	+
	4	Wet wipes	+
Semi dry	1	Water	+
	2	Soap and water	_
	3	Wet wipes	+
Fresh	1	Soap and water	_
	2	Water	_
	4	Wet wipes	_

- Solvent-based Paint

Table 5. Show results of Kastle Meyer test on solvent based tiles after the bloodstains were washed

State of Bloodstains when cleaned	*Row swabbed*	*Type of cleaning*	*+/−*
Dry	2	No cleaning	+
	2	Water	+
	2	Wet wipes	+
Semi dry	4	Water	+
	3	Soap and water	_
	2	Wet wipes	+
Fresh	1	Soap and water	_
	2	Water	_
	4	Wet wipes	_

3.2. Luminol test on Painted Tiles

The luminol test was performed in a black room. Based on Bily's and Maldonato's [8] work positive reaction from the luminol was expected to occur with a maximum of eight layers of

water-based paint. The experiment conducted however received positive reactions up to seven layers with the water-based paint. This difference may be accounted to many variations in the experiment done when compared to Bily and Maldonado's [8] experiment. First in the literature, sections of walls (30x30) *cm* were used to analyse the blood spatter made with an impact spatter apparatus, in contrast to this work where tiles of (99x99) *mm* and (150x150) *mm* used in this experiment to analyse the blood drops that were made with pipettes. The use of a large area would make it easier to observe the results obtained after luminol was applied. Second, the bloodstains not cleaned prior to the application of paint would provide a more concentrated amount of blood on the tiles for the luminol to react with. The action of cleaning in addition to reducing the concentration of blood, it also spreads blood particles along the surface of the tile that was cleaned. Third, the brand of the water-based paint used may have been different. Providing a more uniform or covering layer when applied.

- Water-based paint
  a) Dry

The photograph of the tile cleaned when the bloodstains were completely dry (figure 2) showed the least amount of luminescence comparing with the semi-dry and fresh cleaning. Also this result is in contrast with the results obtained in the Kastle-Meyer test, as can be seen in table 6. In this tile it is possible to observe a stronger luminescence in the area where no cleaning was used, the row cleaned with soap is the one with lower luminescence.

Table 6. Results of water-based tiles cleaned when bloodstains were dried

Number of layers	**Type of cleaning**			
	Soap	***Water***	***Wet wipes***	***No cleaning***
No paint	+++	+++	+++	+++
1	+++	+++	+++	+++
2	++	++	++	++
3	++	++	++	++
4	+	+	+	+
5	+	_	_	_
6	+	_	_	_
7	_	_	_	_

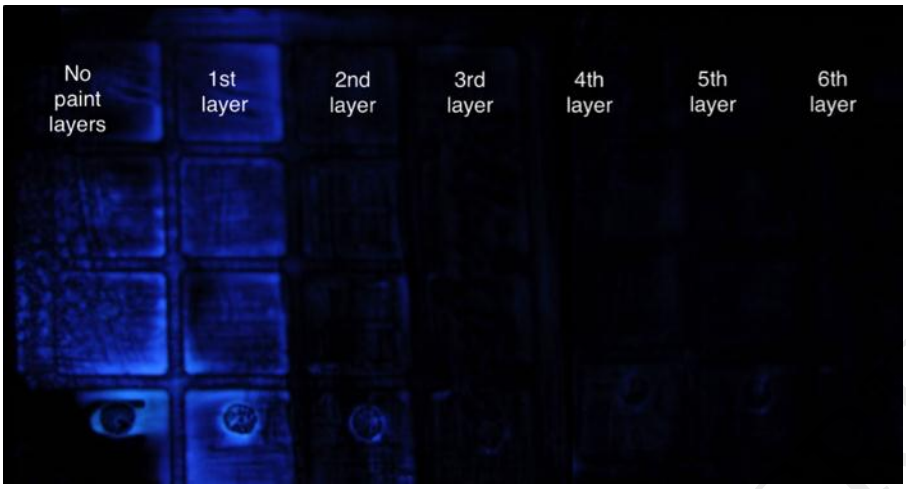

Figure 2. Shows the luminol reaction in the water-based tiles washed when the bloodstains were dried

b) Semi-dry

More luminescence was obtained when the bloodstains was cleaned semi-fresh, visible in figure 3 than when it was completely dry (figure 2). This persisted with the increase of the number of layers. The luminescence was still present with the 7^{th} coat of paint, it was very low intensity and the camera was not able to capture the amount of light emitted during the reactions. With the 8^{th} layer of paint no luminescence was observed in all types of cleaning. In the table 7, the 7^{th} layer appears negative even if it was a positive result. This was because during the experiment it was decided to indicate positive results only those ones that were able to be captured by the camera, since in the image is not possible to observe any light for that layer the result is negative.

Table 7. Results from luminol reaction with water-based tiles washed when the bloodstains were semi-dried

Number of layers	**Type of cleaning**			
	Soap	***Water***	***Wet wipes***	***No cleaning***
No paint	+++	+++	+++	+++
1	+++	+++	+++	+++
2	+++	+++	+++	+++
3	++	++	++	++
4	++	++	++	++
5	+	+	+	+
6	+	+	+	+

7	–	–	–	–

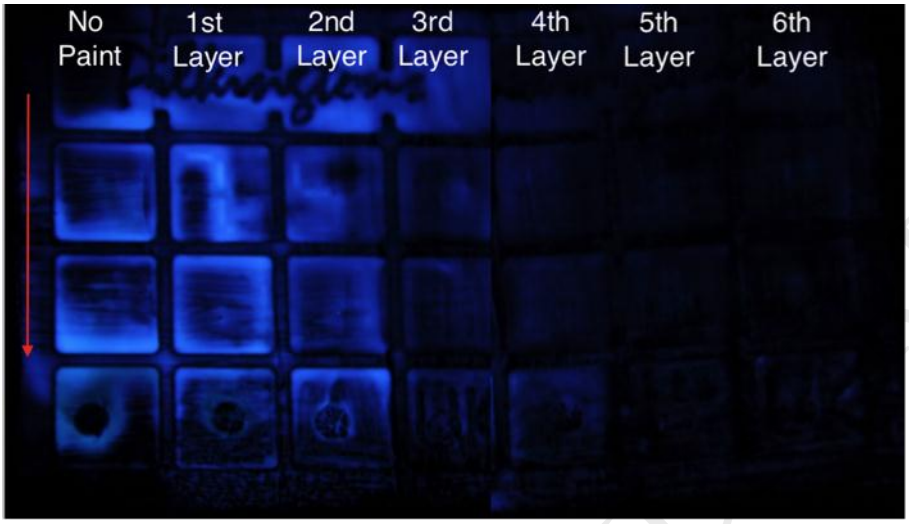

Figure 3. Luminol reaction on water-based tiles, the bloodstains were cleaned when semidry

c) Fresh

Table 8. Shows results obtained when luminol was sprayed on water based tiles cleaned when bloodstains were fresh

Number of layers	**Type of cleaning**			
	Soap	***Water***	***Wet wipes***	***No cleaning***
No paint	+++	+++	+++	+++
1	+++	+++	+++	+++
2	++	++	++	++
3	++	++	++	++

4	+	+	_	+
5	+	+	_	+
6	_	_	_	+
7	_	_	_	_

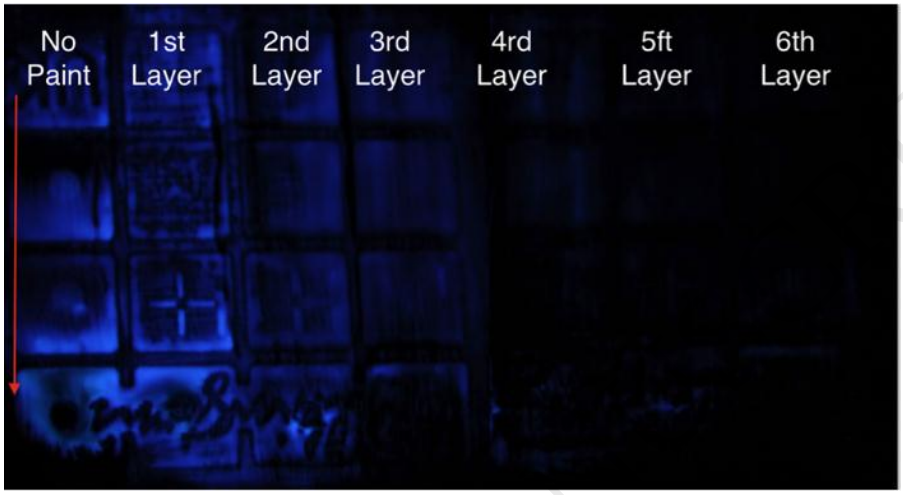

Figure 4. Shows the luminol reaction in the water-based tiles that where cleaned when the bloodstains were fresh

The different cleaning methods did not show varying results among each other and for all the tiles the intensity of chemiluminescence decreased as more layers were applied. It was also observed that among the ridges of the tile the amount of luminescence was higher; this could mean that a higher amount of blood was deposited during cleaning.

It was still possible to observe the bloodstain pattern on the sections which was not covered by paint, which was not visible on the other images (figure 4). This bloodstain pattern was lost with the application of the first layer of paint. It is also possible to affirm from the results that the amount of light differed in the states of cleaning. In general the images obtained in this part of the experiment were really clear and showed exactly what was seen at the time of the experiment. The reaction time was approximately ten minutes before the luminescence faded completely. The luminescence within the number of layer was homogeneous.

Adair [11] obtained similar results, he was able to observed blood in areas up to two layers of paint, in their experiment they did not clean the blood. In fact in the article the author spoke about a light brown colour, which was the effect of the blood under the paint. In their experiment with the application of the third coat of paint the blood was no longer visible. In Adairs [11] experiment the detection of blood was performed with the Polilight^{TM} .

- Solvent-based paint

The reaction of blood with luminol on the solvent-based paint tiles was not as homogeneous as

the reaction observed on the water-based tiles. The smother texture of the solvent-based paint may have been the cause for the difference in the reactions. For all the tiles positives results were observed for the first layer of paint, then varied with the different drying and cleaning methods. The total reaction time of the luminol on the solvent-based tiles was approximately 8 to 9 minutes.

a) Dry

The tiles that were cleaned when the bloodstains were dry showed more luminescence than semi-dry and fresh ones. Positive results were observed up to the first layer of paint, however some dots of luminescence were recorded on the third layer of paint, this may have been the result of an imprecise application of the solvent-based paint on the tile.

Table 9. Shows solvent based tiles cleaned when bloodstains were dry

Number of layers	**Type of cleaning**			
	Soap	***Water***	***Wet wipes***	***No cleaning***
No paint	++	+	+	++
1	+	+	+	++
2	_	_	_	+
3	+	_	+	_
4	_	_	_	+

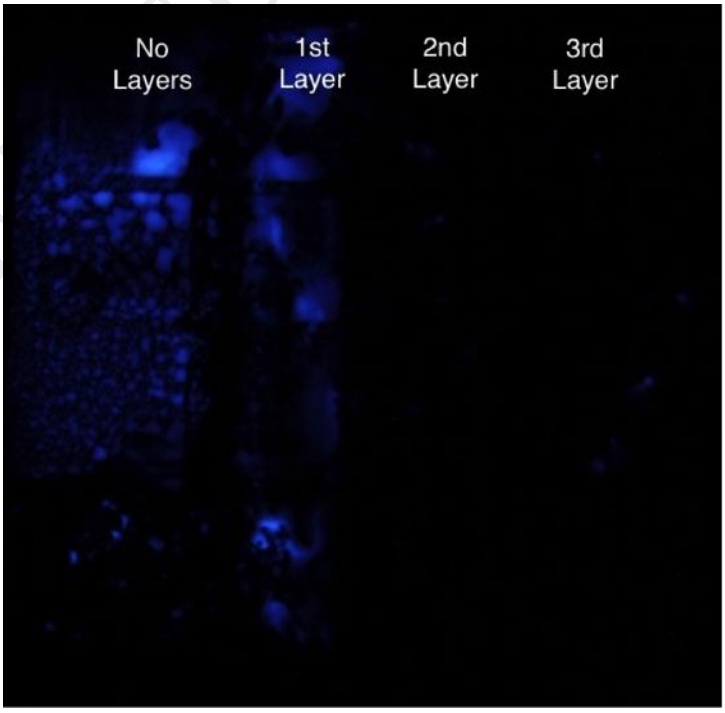

Figure 5. Shows how solvent-based tiles cleaned when the Bloodstains were dry react to luminol

b) Semidry

In the figure 6 it is possible to observe some luminescence in the area of the third layer, like in the tile above, this might have been because of an imprecise application of the paint. Due to the positive reaction of luminol with blood on the area of the third layer, a fourth layer of paint was applied. With the fourth layer no luminescence was observed.

Table 10. Shows the results obtained from the luminol reaction with solvent- based tiles cleaned when bloodstains were Semi dry

Number of layers	Type of cleaning			
	Soap	***Water***	***Wet wipes***	***No cleaning***
No paint	++	+	+	++
1	+	+	+	+
2	+	_	_	+
3	+	_	_	+
4	_	_	_	_

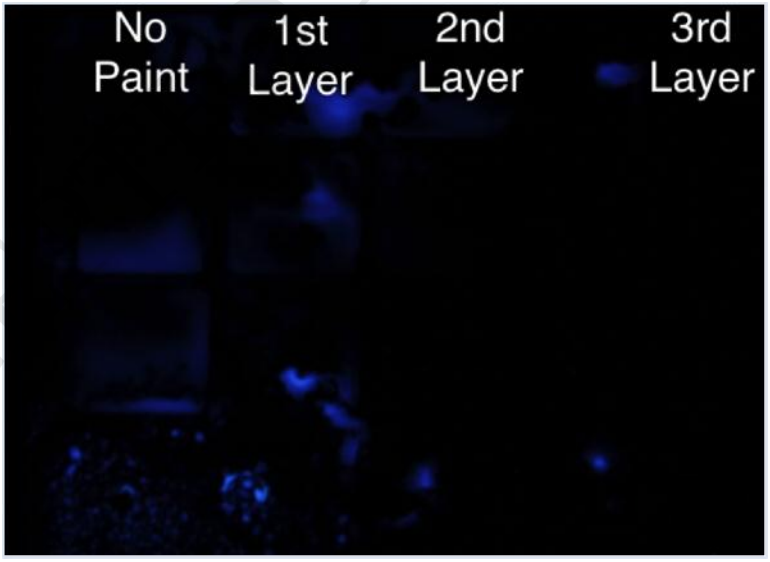

Figure 6. Shows reaction of luminol on solvent-based tiles cleaned when blood- stains were semidry

c) Fresh

Table 11. Shows the results obtained from the luminol reaction with the solvent- based tiles cleaned when the bloodstains were fresh

Number of layers	Type of cleaning			
	Soap	***Water***	***Wet wipes***	***No cleaning***
No paint	+++	+	+	++
1	+	+	+	++
2	_	_	_	+
3	+	_	+	_
4	_	_	_	_

The tiles that were cleaned when the bloodstains were fresh (shown in figure 7), more chemiluminescence was observed in the area of the tile that had no cleaning. The other areas showed almost no luminescence at all. This means that the smoothness of the tile had a bigger influence on this type of tiles because the bloodstain deposited on the tile stayed on the tile for two minutes before being cleaned, not allowing the same amount of blood to deposited on the tile like in the tiles that had 15 minutes and 1 hours (see figure 6 and 5).

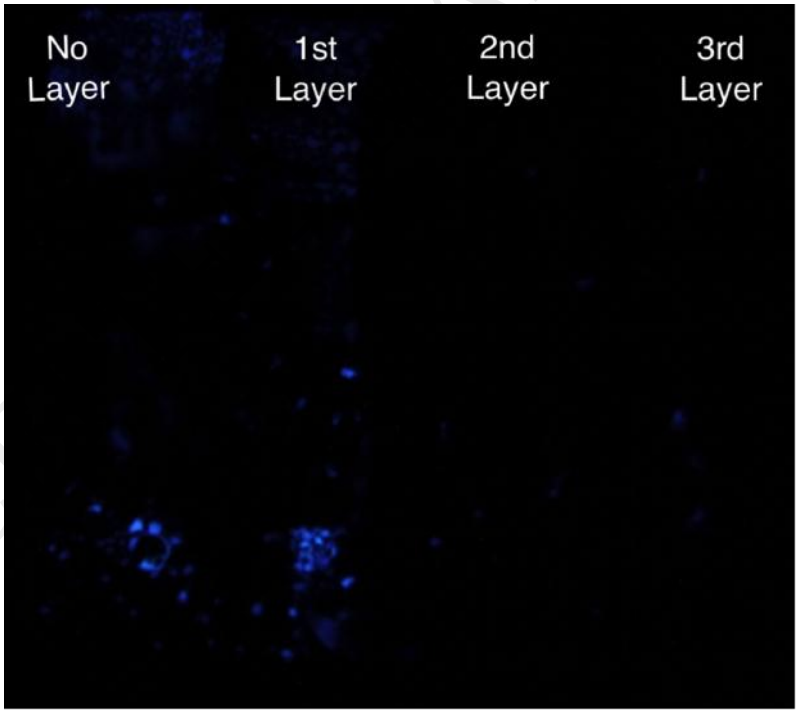

Figure 7. Shows reaction of luminol with the Solvent-based tiles that were cleaned when the bloodstains were still fresh

The following table (table 12) was obtained calculating the means of the observations, using the table 3. The means were used to create the graph shown on figure 8. It shows the trend of the intensity of light when the number of layers of paint increases.

Table 12. Mean of the values obtained in the observations of the luminol reaction

Number of Layers	*Water-based*	*Solvent-based*
0	1.5	0.791
1	1.5	0.583
2	1.167	0.167
3	1.167	0.25
4	0.625	0.083
5	0.333	0
6	0.25	0
7	0	0

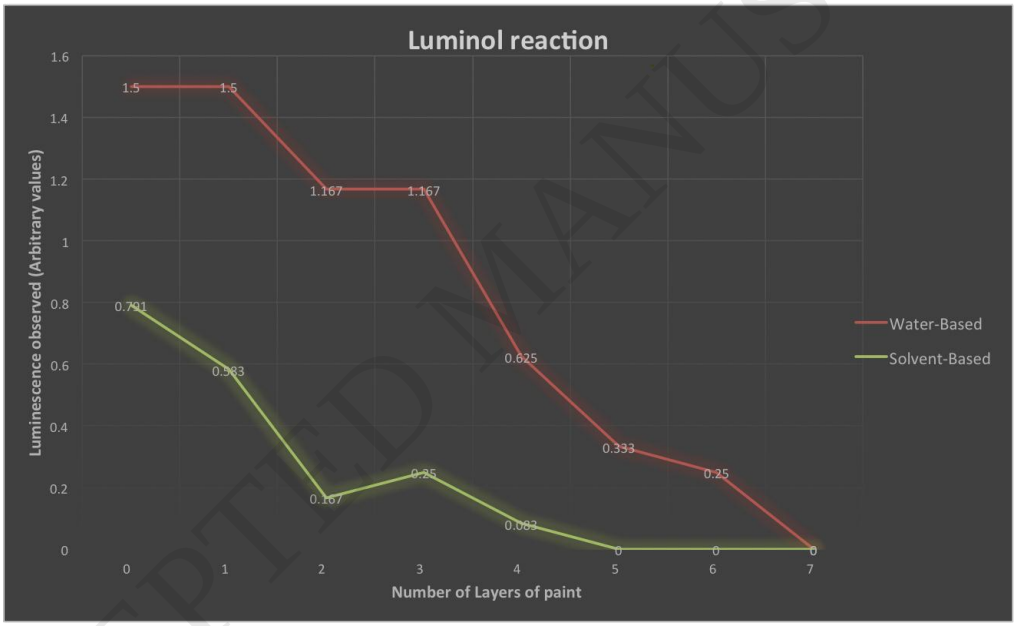

Figure 8. Shows intensity of light emitted at the increase of the number of layers of paint

3.3. Bleach Test

During the progress of the bleach test it was noticed that the reaction of luminol was completely different in all the experiments. When the tile was cleaned with bleach and no blood was present in the tile the reaction was very fast. On the tile that blood was present and washed with other methods the reaction of luminol was very intense, even with the layers of paint, and it lasted for more than 10 minutes. On the tiles with blood but that were cleaned with bleach the reaction was a mix of both of the reactions explained earlier, it was not intense as the reaction with blood but it did last for almost 8 minutes. The results obtained in the bleach reaction with luminol is less intense than washed blood and luminol itself. These results are in concordance with the result produced by Quickenden and Cooper [12], in which they showed that the reaction of blood at two different concentrations with luminol emitted more light than the

reaction of sodium hypochlorite with luminol. An interesting experiment in which the drying time of the bleach is tested on porous surfaces and the effect on the luminol test. They were able to observe that porous surfaces such us brick and cotton fabric after one day of drying had a positive reaction indoors, when outdoors only the brick had a positive reaction after a day of drying [13].

Table 13. Shows results from the bleach study

Type of paint	***Presence of blood Yes/No***	***Luminol reaction Yes/No***	***Surface of tile that reacted to luminol***	***Type of reaction/Time of reaction***	***Number of layers of paint applied after cleaning***
Water-based	No	Yes	Entire tile	Not very present; max 2 minutes	None
Solvent-based	No	Yes	Side of tile	Sparkle, seconds	None
Water-based	Yes	Yes	Entire	Faded luminescence; 8 minutes indication of presence of blood	None
Water-based	Yes	Yes	Entire	Faded luminescence; 6-8 minutes indication of presence of blood	1
Solvent-based	Yes	Yes	Zones	Very faded; seconds	None
Solvent-based	Yes	Yes	Zones	Very faded; seconds	1

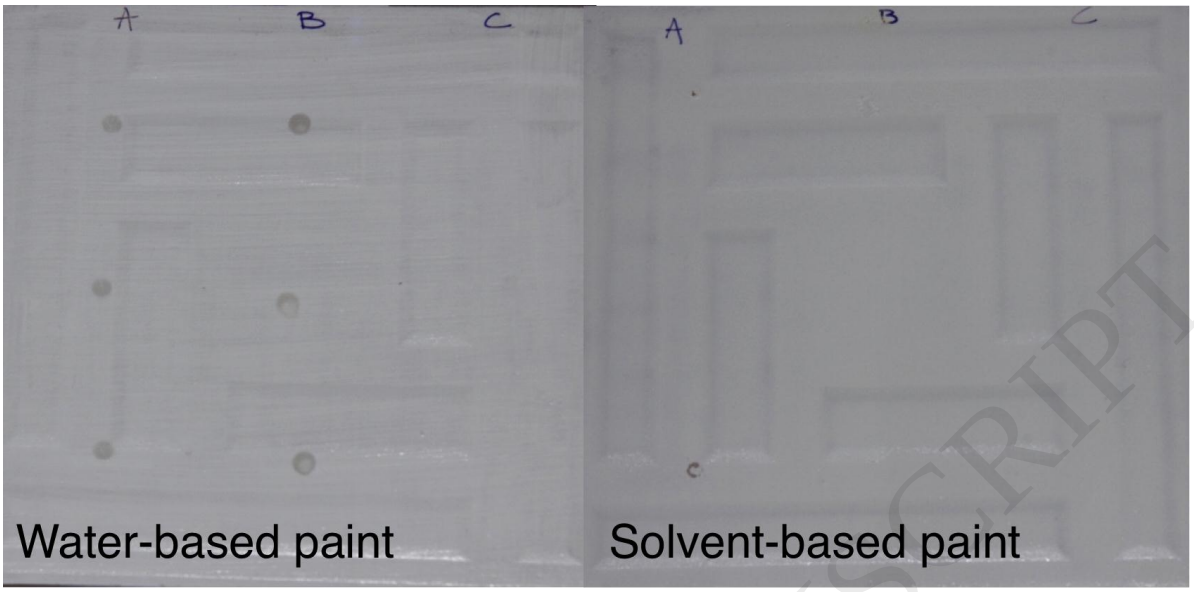

Figure 9. Shows tiles after the cleaning with bleach

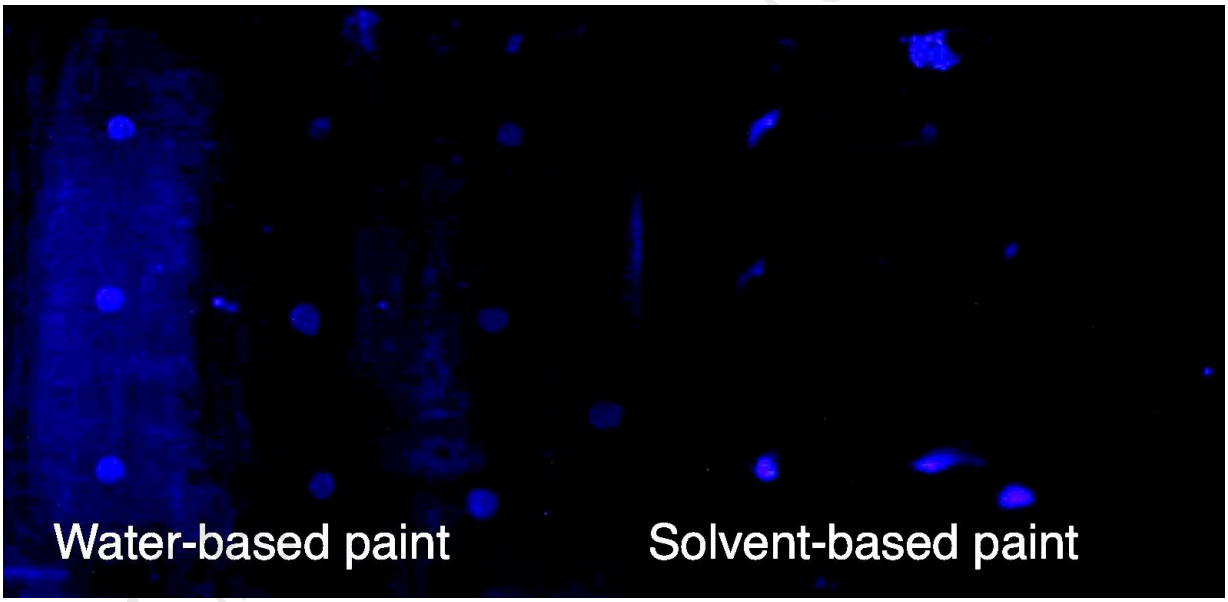

Figure 10. Shows reaction of luminol after blood and cleaning with bleach

3.4. Microscopical Study of the surface of the tiles

This study was performed in order to understand if the surface of the two types of paints was different and to understand also if the surfaces itself contribute the differences described in the sections above. The tiles used were the control tiles, that had no blood on them but were washed with bleach. On this tile a drop of defibrinated horse blood was applied and after two minutes it was wiped with tissue. It was possible to observe from the figure 11 (left) that the surface of the water-based paint is heterogeneous, it formed bubbles that burst when the paint dries and fills up with blood when it is deposited. The figure 11 (right) has a homogeneous surface and a lower number of bubbles, when the paint was deposited it created striations not visible in the

figure 11 (left). Both the figures were taken at the same magnification, with the same microscope. Another reason the water-based paint showed more chemiluminescence with luminol when blood was present could be due to the number of bubbles present on the surface of the tile.

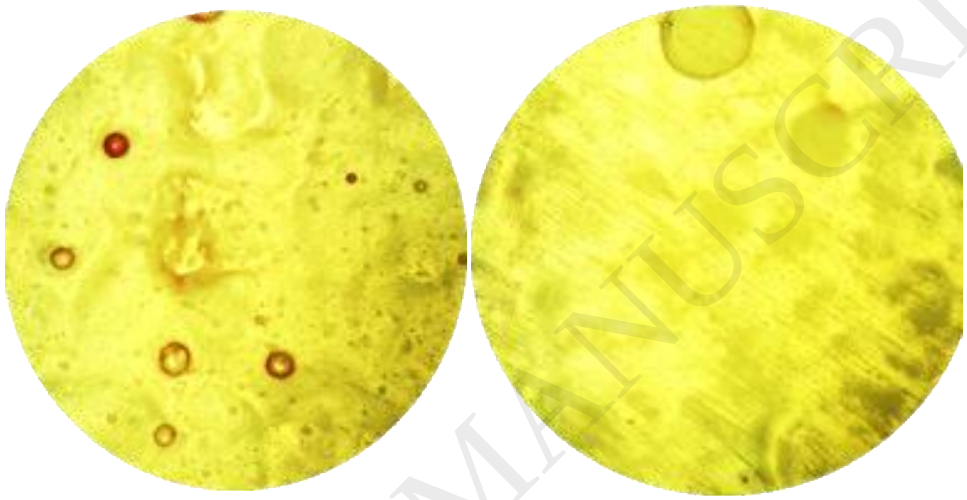

Figure 11. Left: Shows surface of water-based paint at the microscope. Right: Shows surface of solvent-based paint at the microscope

4. Conclusions

In conclusion water-based paint has a lower concealing power than the solvent based paint. This can be affirmed from the different tests performed during the experiment. The differences in the reactions may have been due to the following. Firstly the solvent based paint being thicker than the water-based paint permitted a lower number of layers of paint in order to conceal the traces of blood. Secondly the microscopical study showed that the water-based paint formed bubbles along the surface, while the solvent-based formed striations when blood was applied.

Bily and Maldonato [8] suggested areas for further investigation, in this work we covered : (1) the application of paint through a sprayer instead of the use of a roller or brush, improvement in the photography and a study of different types of paints. The authors of this study decided to use a brush and not a sprayer because in common household painting a brush is much more commonly used than a paint sprayer. (2) This work managed to obtain very good and clear images from the chemiluminescence of luminol and blood. (3) The comparison of paints that are most commonly used .

Novelty Statement

The bloodstains on walls and floors are often removed by cleaning, in some cases the surfaces are painted by the perpetrator after committing a violent crime in order to hide the crime that has occurred. The study hereafter extends and deepens on previous researches by investigating the detectability of horse bloodstains on painted ceramic tiles as a function of the number of layers of paint. In this study luminol was used as a reagent to detect the bloodstains. The study focuses on two types of paints: water based and solvent based paint. This study also investigates the effectiveness in reducing the detectability of bloodstains on ceramic tiles using four different cleaning methods pure water, soap with water, wet wipes, and bleach. In the experiment the bloodstains were cleaned at various intervals of time after the deposition (two minutes, fifteen minutes and one hour).

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