Abstract:

This study evaluates a selection of techniques to aid in the detection or enhancement of bloodstains on dark surfaces, particularly in relation to the examination of items submitted to the Biology laboratory. Techniques were assessed and compared according to their ease of use, effectiveness on different surface types and any effects on subsequent testing, DNA recovery and pattern recognition. The result is the implementation of selected techniques into the Biology lab for the detection of blood on dark surfaces, pertaining to both DNA and Bloodstain Pattern Analysis examinations.

Introduction:

Blood is probably the most commonly encountered bodily fluid at crime scenes and is an important form of evidence[1]. It can identify individuals through DNA, and through Bloodstain Pattern Analysis may; determine the number of parties involved, indicate the movements of those involved and provide information on possible injuries attained.

Locating bloodstains on dark coloured items can be difficult due to the lack of contrast between the colour of the blood and the colour of the receiving surface[2]. The location of blood on an item may be of value for many reasons including; confirming or refuting allegations relating to an offence, sampling for DNA analysis and potential recognition of patterns through BPA. For the bloodstain pattern analyst, visualising the pattern of staining is essential, thus the employment of visual, photographic or chemical techniques to locate or enhance the bloodstains is pertinent to a thorough investigation.

The current method used in the Australian Federal Police Biology laboratory is the orthotolidine screening method. This method involves rubbing a piece of filter paper over the surface of an item, then applying the chemical to see if a reaction is obtained. It may be necessary to complete this process many times over in order to successfully identify the location of any blood. The process is time consuming, destructive to bloodstain patterns and may lead to a loss of evidence. Luminol has been employed during scene examinations, however has rarely been used in the laboratory environment due to safety concerns.

Method/Materials: (Refer to Appendix 1 for further details on method preparation)

Neat, freshly drawn blood was applied to a range of dark coloured surfaces and allowed to dry. Surfaces included cotton, denim, polyester, fleece, wool, cotton print, carpet, boot and knife. Blood was also applied to white cotton to allow testing of the effect on pattern recognition.

Additional samples of cotton, polyester, wool and denim were washed to allow for sensitivity testing. The samples were washed using a normal domestic process aiming to replicate casework items as closely as possible.

Following application of the detection and enhancement techniques, samples were taken for subsequent presumptive, confirmatory and DNA testing. Samples were subjected to the o-tol presumptive test, ABAcard HemaTrace® & RSID™-Blood confirmatory tests and DNA analysis. DNA analysis was performed as following; extraction using the Qiagen Symphony, quantification using Quantifiler Real-Time PCR, amplification using AmpF/STR Profiler Plus™ and analysis on the ABI 3130xl.

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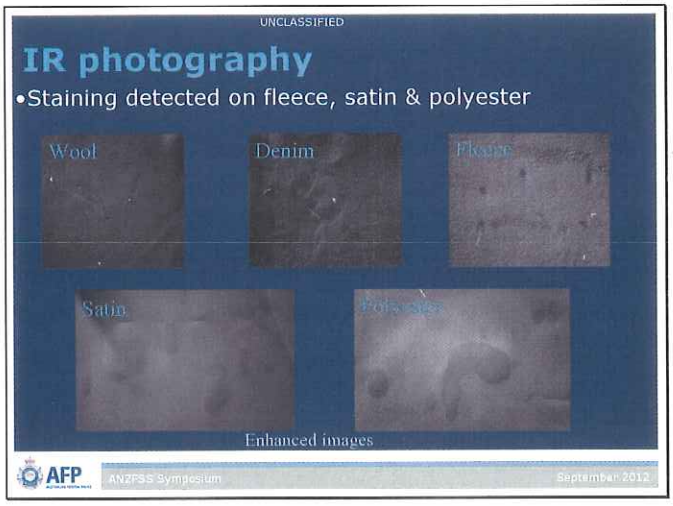
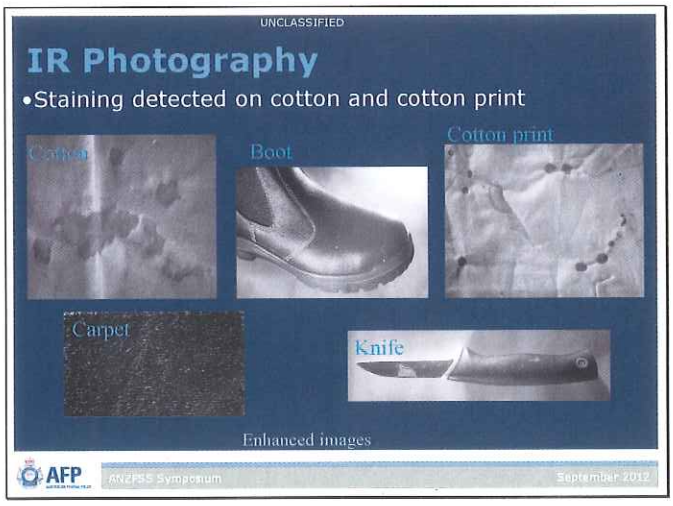
Results:

IR photography;

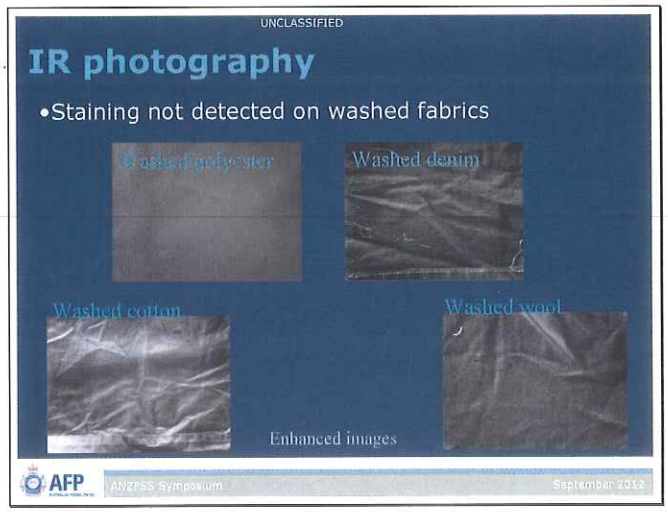
Contrast was achieved on satin, polyester, cotton, cotton print and fleece.



Contrast was not achieved on denim, wool, boot, carpet or knife.



There was no staining detected on any of the washed fabrics.



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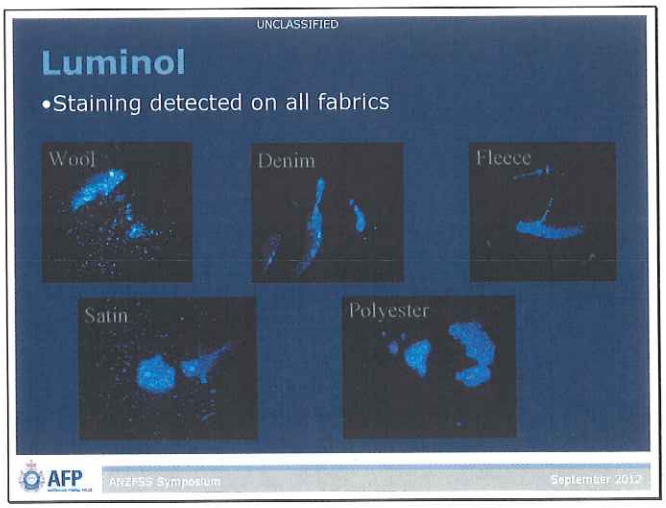
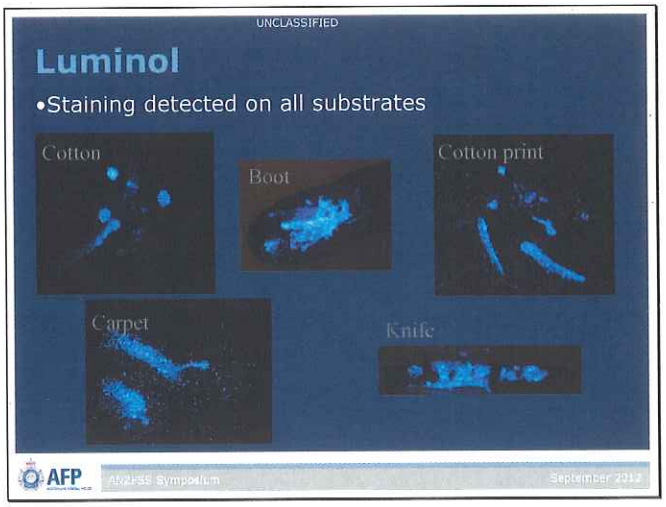
Luminol;

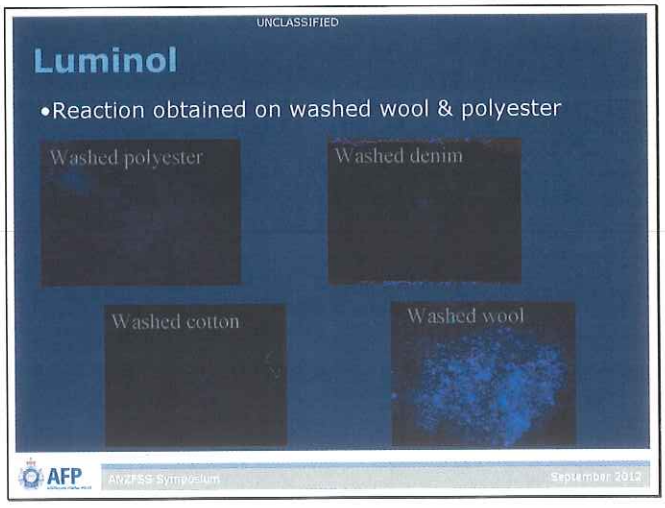
Luminol detected staining on all porous surfaces.



Luminol detected staining on all non-porous surfaces, although it did result in the running of stains.

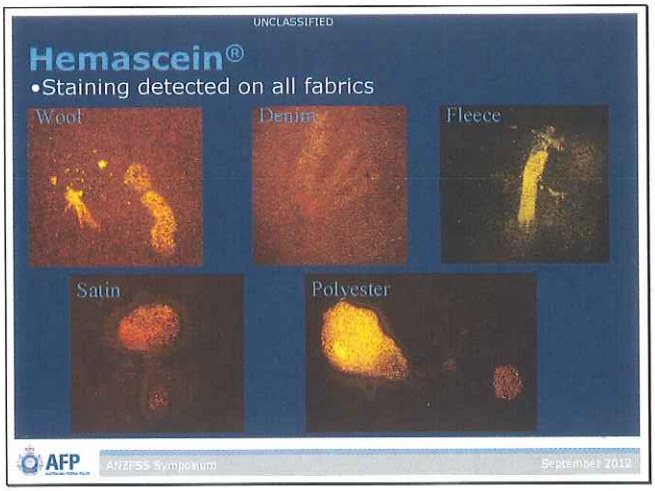


Luminol reacted on the washed polyester and wool samples.

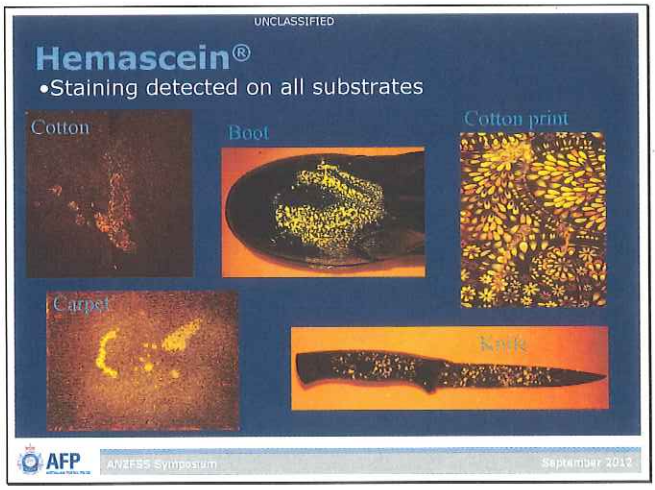


Hemascein®;

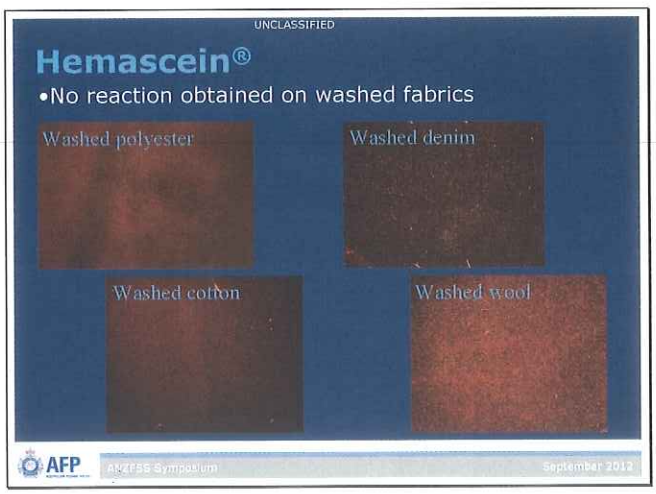
Hemascein® detected staining on all porous surfaces. Slight background fluorescence was observed on the denim sample and although a result was obtained on the printed cotton, insufficient contrast was achieved.



The Hemascein® reagent detected staining on all non-porous surfaces with less running of the staining when compared with luminol.

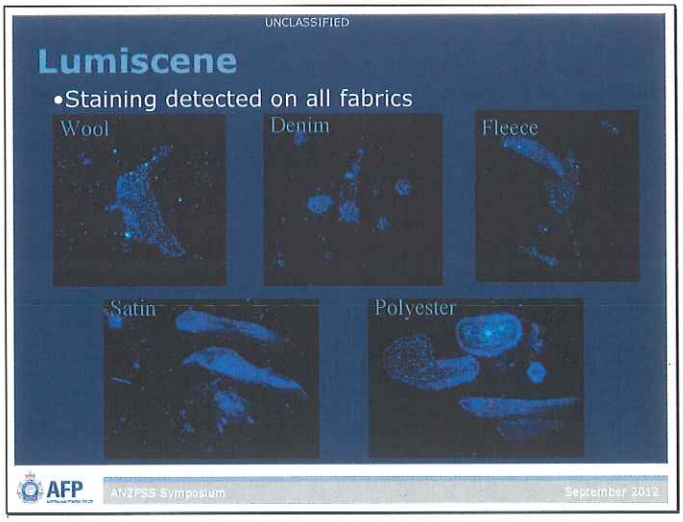


No reaction from Hemascein® was obtained on the washed fabrics.

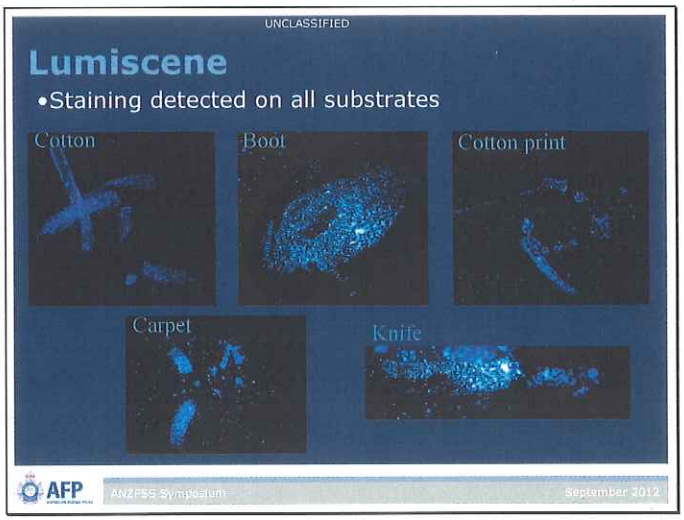


Lumiscene;

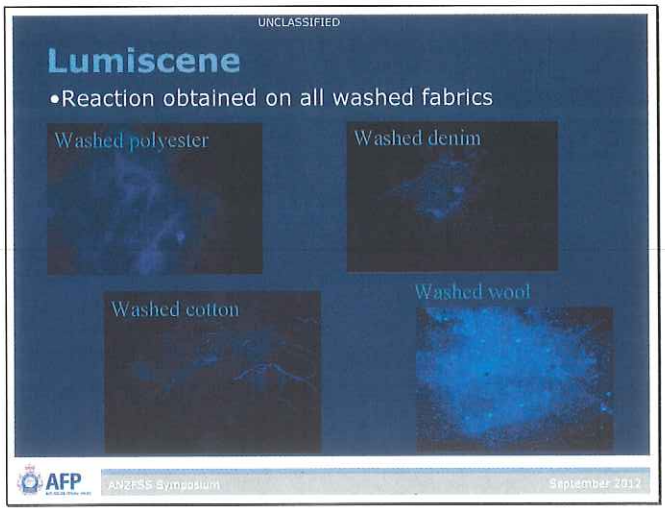
Lumiscene detected staining on all porous surfaces.



Lumiscene detected staining on all non-porous items with less running than luminol.

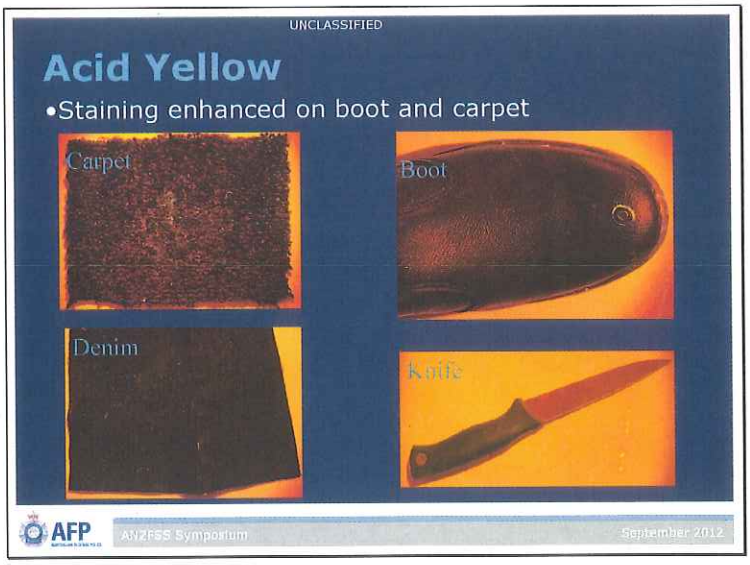


Lumiscene reacted on all washed fabrics.



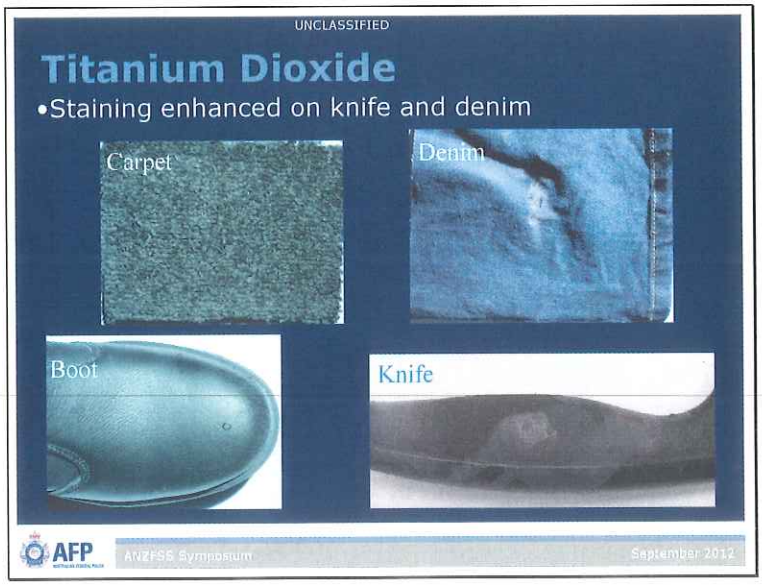
Acid yellow;

Staining was enhanced on the carpet and boot; however no results were obtained from the knife or denim.

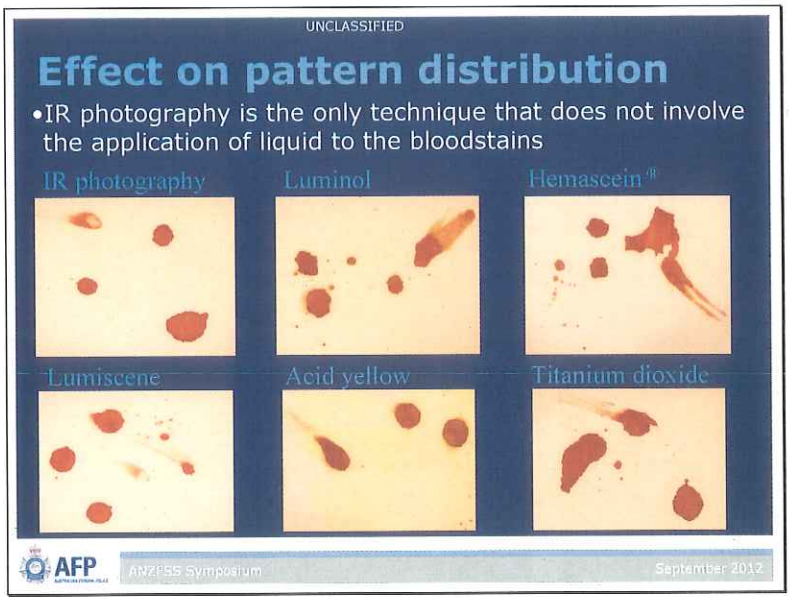


Titanium Dioxide;

Some enhancement of staining occurred on the knife and a reaction on denim was observed. No results were obtained from the boot or carpet.



Pattern recognition;



Infrared photography has no effect on the pattern distribution.

Luminol caused a moderate amount of running of the staining. Hemascein® and Lumiscene resulted in less running of the staining when compared with luminol, although the application of the hydrogen peroxide in the Hemascein® method cause some 'bubbling' to occur. Lumiscene resulted in the least effect on pattern recognition of all the chemical detection methods tested.

Acid Yellow and Titanium Dioxide had an effect on the pattern distribution due to the application of liquid to the porous surface. This result is interpreted with caution as both techniques are best suited to non-porous surfaces.

Figure 1: Summary of results

Method	Ease of use	Optimal surface type	Effect on pattern distribution	Successful subsequent testing	DNA profile obtained
IR photography	Moderate - complex	Various	Nil	✓	✓
Luminol	Moderate	Porous & Non-porous	Moderate	✓	✓ #
Hemascein®	Moderate	Porous & Non-porous	Slight	✓	✓ #
Lumiscene	Easy	Porous & Non-porous	Slight	✓	✓
Acid Yellow	Complex	Non-porous	*Moderate	✓	✓
Titanium Dioxide	Easy	Non-porous	*Slight	✓	✓

* Tested on porous surface

Partial profile obtained from washed cotton sample

Discussion:

The Infrared photographic method for the detection of blood staining on dark substrates proved to be an effective method with the advantage of being non-destructive to the staining. This method may not be suited to all substrates depending on the material composition and absorption or reflectance of infrared light. The effectiveness of this technique on particular surfaces cannot be routinely predicted, (i.e. porous or non-porous); however it can be simply identified through viewing the sample with the IR camera.

The Nikon 'live-view' feature simplifies the searching process and following image capture, image enhancement software is utilised to increase the contrast (i.e. Adobe Photoshop).

In order to locate any blood staining on dark surfaces, it may be necessary to employ a method of detection. The detection of blood relies upon a technique which can be applied to the entire surface of an item producing a reaction to indicate the location of possible bloodstains. These methods are generally of a chemical nature and involve the application of a reagent to the item. The application of liquid to a bloodstain will generally have some altering affect on the shape, particularly on porous surfaces. As such, interpretation of any bloodstain patterns following the use of these methods should be made with caution.

Luminol is perhaps the most recognised chemical detection method for blood, however advancements have resulted in products such as Hemascein® and Lumiscene, offering an alternative.

Luminol is a recognised technique for the detection of blood. It is highly sensitive, cost effective and moderately easy to use. Luminol can be mixed in required volumes which is advantageous when examining items in the laboratory, however the life of the working solution is quite short (2-4hrs). The resulting luminescence is short lived and may require repeated application of reagent, of which is a potential issue for DNA recovery. The application of the liquid causes running of stains and may dilute the sample, potentially affecting DNA analysis.

Hemascein® is an effective technique for the detection of blood providing a reaction which was relatively easy to visualise and record. The mixing of the solution is simple and has the advantage of only using the required amount of reagent for any given examination. The shelf life of the unmixed reagent is considerable and the life of the working solution proved to be in excess of 1 week (in this study), providing a cost effective method. The ABA spray proved to be an effective mechanism of delivery and little reagent was required to achieve a result. The application of Hydrogen Peroxide caused some 'bubbling' in turn affecting the integrity of the staining. There was no effect on the subsequent testing, however there was a decrease in the DNA yield from the washed cotton sample.

Lumiscene is a simple, effective method of detection for blood on dark surfaces. The reagent, as supplied, requires little preparation and the results are easily visualised and recorded. It is a sensitive technique applicable to most surface types. The shelf life of the reagent prior to mixing is considerable and the life of the working solution exceeded that which was documented (in this study a reaction was obtained 1 week post mixing). The provision of Lumiscene in smaller volumes denotes this to be a cost effective technique for use within the laboratory environment. This method requires only a small amount of reagent to achieve a result, thus a more suited application process would be using a compressed air system.

The chemical enhancement techniques are most suited to the enhancement of detailed marks in blood on non-porous surfaces. These methods are often used to enhance specific areas of an item and not ideally suited to application over a large surface as a detection method.

Acid yellow is a technique frequently utilised in the enhancement of fingermarks in blood on dark, non-porous surfaces. The three solutions required for this technique have an extended shelf life making this a cost effective technique to have available. This method was applied using a pipette in this study; however application via a spray mechanism would be more effective, applying less reagent to a larger area. Despite reacting with the proteinaceous component of the blood[3], subsequent testing and DNA analysis was successful.

Titanium dioxide is a simple, easy to use technique that can be used to enhance blood on dark non-porous surfaces. The cost of reagents is low and shelf life indefinite. This enhancement method adheres to the latent (non-blood) areas[3] of a fingermark deposited onto a surface. The recommended application method is via pipette or spray device to avoid any unnecessary disruption to the staining. Subsequent testing of the blood and retrieval of DNA was possible following the application of this method.

Conclusion:

The laboratory examination of bloodstains on dark coloured items can provide valuable information to an investigation. Blood, as evidence, may present itself on an assortment of items submitted to the laboratory and as the variation in surface type, colour and composition is infinite, the optimal approach is to have a selection of detection and enhancement techniques available to the examiner. The provision of training and guidance can then enable the examiner to select the most appropriate technique for the given item, ensuring a thorough investigation and optimisation of results obtained.

Pertaining to the examination of bloodstained items within the AFP Biology laboratory, it is recommended that a number of detection and enhancement techniques be implemented for use. These techniques include, but are not limited to; Infrared photography, Lumiscene and Acid Yellow.

IR photography proves to be a valuable screening tool, favoured for its non-destructive nature. It has the distinct advantage of both detecting and enhancing bloodstains present on dark surfaces. Lumiscene is the preferred chemical detection method due to the ease of use, sensitivity, quality of results and successful retrieval of DNA following treatment. Acid Yellow is a reliable method for the enhancement of detailed marks in blood as the integrity of the staining is maintained and subsequent testing and DNA analysis can be successfully conducted.

References:

1. Virkler, K. & Lednev, I., Forensic Science International 188 (2009) 1-17, *Analysis of Body Fluids for Forensic Purposes: From Laboratory Testing to Non-destructive Rapid Confirmatory Identification at a Crime Scene*.
2. McQuisten F., *The Photographic Enhancement of Bloodstain Patterns on Dark Fabric*, March 2006, Canberra Institute Technology
3. Bossers, L.C., Roux, C., Bell, M. & McDonagh, A., Forensic Science International 210 (2011) 1-11, *Methods for the Enhancement of Fingermarks in Blood.*

Appendix 1: Methods and Materials

IR photography;

Nikon D700 with internal UV/IR blocker removed. A HOYA R72 IR filter was used and a light source to provide adequate IR light (Halogen lights). Adobe Photoshop was used to enhance images.

Luminol;

Prepared according to the current AFP protocol using Sodium Carbonate, luminol and hydrogen peroxide. The luminol reagent was applied using a spray bottle. Results were recorded using the Nikon D700 with timed exposure (20-30 sec).

Hemascein®;

Prepared as per kit instructions; Hemascein® powder mixed in water to form the stock solution. The working solution then made from the stock solution + water. The working solution was placed into one ABA spray and 3% hydrogen peroxide placed into the other. The Polilight (440-450nm) was used to visualise, with the Nikon D700 with orange filter to record.

Lumiscene;

Prepared as per kit instructions; two activation tablets placed into the solution, mixed and sprayed using spray nozzle provided. Results were recorded using the Nikon D700 with timed exposure (10-20sec).

Acid Yellow;

Three part method. 1. Fixative solution consisting of sulfosalicylic acid & water, 2. The working solution of acid yellow dissolved in water, ethanol and acetic acid and 3. The wash solution made up of water, ethanol and acetic acid. This method was applied via pipette, visualised with the Polilight (450nm) and recorded with the Nikon D700 with orange filter.

Titanium dioxide;

Approx. 10g titanium dioxide powder mixed into 100ml Methanol. The reagent was applied via a spray bottle and rinsed with methanol. Visualised with white light and recorded with Nikon D700.