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Chemical enhancement of footwear impressions in blood on fabric — Part 2: Peroxidase reagents

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ABSTRACT

This study investigates the optimisation of peroxidase based enhancement techniques for footwear impressions made in blood on various fabric surfaces. Four different haem reagents: leuco crystal violet (LCV), leuco malachite green (LMG), fluorescein and luminol were used to enhance the blood contaminated impressions.

The enhancement techniques in this study were used successfully to enhance the impressions in blood on light coloured surfaces, however, only fluorescent and/or chemiluminescent techniques allowed visualisation on dark coloured fabrics, denim and leather. Luminol was the only technique to enhance footwear impressions made in blood on all the fabrics investigated in this study.

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1. Introduction

Heme-reacting chemicals react with the heme group in haemoglobin present in blood. These chemicals, also known as peroxidase reagents, are colourless dyes that are oxidised to form a coloured product [1,2]. Compared to protein stains, these reagents do not react with body fluids apart from blood, however, trace amounts of blood in urine, saliva and other body fluids will be detected [3]. Some peroxidase reagents have been reported to react with vegetable peroxidases [4] or any substance with peroxidase activity [3]. Furthermore, background staining might occur where the background is slowly oxidised to the same colour as the blood enhanced impression. Examples of heme-reacting chemicals include leuco crystal violet (LCV), leucomalachite green (LMG), benzidine, tetramethylbenzidine (TMB), phenolphthalein, fluorescein and luminol.

Haemoglobin exhibits peroxidase activity by catalysing the oxidation by peroxide of a number of organic compounds to yield coloured compounds [5]. As a result, these reactions are also known as catalytic tests. The general peroxidase reaction is as follows:

$$AH_2 + ROOH \rightarrow A + ROH + H_2O$$

where AH_2 is the electron donor and ROOH is the peroxide. The simplest peroxide is hydrogen peroxide where R = H.

Addition of hydrogen to the delocalised systems of dyes usually interferes with the absorption of visible light [6]. Fig. 1 illustrates the oxidation of colourless leuco crystal violet to the purple coloured crystal violet. The leuco compound is less conjugated leading to loss of colour whereas the positive charge on the dimethyl group in crystal violet is able to delocalise over the whole molecule, imparting a bright purple colour. Organic aromatic molecules, such as crystal violet, with conjugated bonds and large systems of delocalised electrons can exhibit visible colour and permit molecular binding to a material [6–8].

Enhancement of blood impressions with LCV and LMG utilise hydrogen peroxide (H_2O_2) and sodium perborate ($NaBO_3.H_2O$) respectively as the reducing agents to create an almost instantaneous colour change. Fresh solutions need to be prepared prior to use as both reagents are light and heat sensitive. Grodsky et al. [9] reported that the use of sodium perborate instead of hydrogen peroxide in the LMG formulation provided a great improvement in the reaction. The reaction between LMG and blood results in a green colour whereas the reaction of LCV and blood results in a vivid purple colour, though it has been observed that although LCV provided better contrast than LMG, it may not be as sensitive as the protein stains acid black 1, acid violet 17 and benzoxanthene yellow [10]. Recent research [11,12] has reported the development of a simple, quick, one-step method for the recovery and enhancement of blood contaminated footwear impressions using nylon membranes, previously impregnated with LMG or LCV to provide excellent enhancement and lifting simultaneously.

The formulations of LCV and LMG have the advantage of incorporating the fix, allowing the blood impression to be fixed and enhanced at the same time [13,14]. Nevertheless, diffusion of

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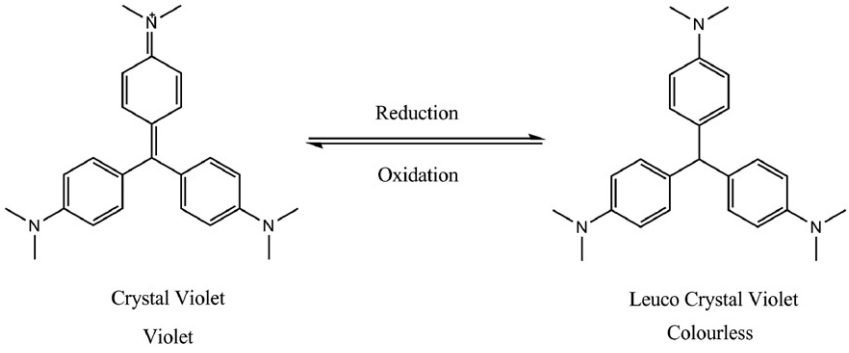

Fig. 1. The reduction and oxidation of crystal violet and LCV.

impressions in blood is a possibility using this one-step process as the fixation process is not instantaneous. Furthermore, LCV enhanced impressions can potentially fluoresce and luminesce using different excitation wavelengths [15]. Crystal violet, formed during the reaction of LCV with blood, fluoresces in the wavelength region 450–800 nm depending on the solvent it is dissolved in [16]. Crystal violet is excited by using a green-yellow source (503–591 nm if using a Mason Vactron Quaser 40) and viewed with a red filter (593 nm). A de-staining procedure is not necessary for peroxidase reagents, but if heavy staining occurs, for example on porous surfaces, it has been suggested that the item can be rinsed with water for 2 to 3 min after the reagents have been applied [13]. Theeuwen et al. [17] have reported LCV as an excellent technique specifically for the enhancement of footwear impressions in blood.

Fluorescein has a chemical structure similar to sulfonated protein stains such as acid black 1 (AB1) and acid yellow 7 (AY7). It is, however, applied as a heme-reagent for the detection of blood with the additional advantage of green fluorescence when illuminated with light at a wavelength of 450 nm. The application of fluorescein for the detection of blood in forensic science has been developed by Cheeseman [18–20]. Fluorescein is soluble in alkali hydroxides and carbonates at room temperature and is reduced from fluorescein to fluorescin in alkaline solution over zinc. In contact with blood, fluorescin is quickly oxidised back to fluorescein by the catalytic activity of the heme in the presence of hydrogen peroxide [1,5]. Several studies have shown that there is no interference with the fluorescein reaction and the subsequent DNA analysis [21–24].

Luminol has been used for many years as a presumptive test for the detection of blood and various formulations of the reagent have been reported in the literature. There are also commercial formulations now available, most notably produced by Bluestar®. Luminol utilises the peroxidase-like activity of the heme group in blood for the production of light and as such is a chemiluminescent test. Sears et al. [10] suggest that this method may be useful for the detection of footwear impressions in blood on dark and patterned carpets but reported that diffusion of the fine detail can occur in the enhancement of some blood-contaminated marks such as fingerprints. Other research has highlighted the sensitivity of luminol where blood was detected through eight layers of paint [25]. Luminol requires the use of specialised photography for visualisation and different formulations of luminol have varying durations of light levels which can sometimes be disadvantageous.

The results obtained from luminol must be interpreted carefully since the reagent is known to give false positives, mainly for bleach which is commonly used for crime scene cleanup [26–33], however, it has been suggested that experienced users of the reagent can distinguish between the reaction of luminol with blood and bleach [1,27,34,35]. More recently it has been shown that luminol's reaction with bleach is greatly varied depending on the formulation of reagent used, the concentration and origin of the bleach and the period of time the bleach has had to dry [36]. Luminol is a useful technique for the enhancement of latent bloodstains and does not interfere with the analysis of DNA [37]. Luminol has been demonstrated to produce false positives with a variety of materials including household products (e.g. oil-based paints, alkyd varnish), food products (e.g. leek, ginger, carrot) and chemical products (e.g. $CuSO_4$, $FeSO_4$) [18,38,39]. It is thought that ions such as Cu^{2+}, Fe^{2+} and Mn^{2+} catalyse the chemiluminescence reaction of luminol whereas SO_4^{2-} does not. Bluestar® luminol, when compared to other luminol formulations has been reported as producing brighter and longer chemiluminescence, is easier to prepare, visualise and photograph and is more efficient in detecting latent bloodstains after attempted cleaning by both soapy water and bleach [26,27,35,40,41].

While previous studies have reported on the abilities of peroxidase reagents to enhance blood impressions, no work has to date compared the range of currently used reagents on the enhancement of repetitive marks in blood prepared under the exact same conditions and across a variety of fabric types. This study compares the effectiveness of four peroxidase reagents commonly reported in the literature to enhance repetitive marks made in blood on nine different fabric types. The fabrics investigated included natural and synthetic material of a range of colours and porosity.

2. Materials and methods

2.1. Deposition of the footwear impressions and preparation of the test marks

The objective of this work was to compare the ability of various peroxidase reagents to enhance footwear marks, rather than mimic operational conditions normally encountered. As a result, small defects were not introduced onto the new footwear sole. The main aim of this study was to compare the ability of a number of peroxidase reagents for the enhancement of blood on fabrics as no previous systematic study of this nature has appeared in the literature to date. Footwear impressions in blood on fabric were prepared by using a semi-automated stamping device which has been described in a previous publication [42] and the associated publication relating to this study [43]. A stamping force rather than a walking force was used.

Six footwear marks were prepared for all tests undertaken within the study. All impressions were allowed to age for seven days before enhancement with the various peroxidase reagents. Photography of all impressions was performed, using a Canon EOS 300D [sensor size 22.7×15.1 mm (3.42 cm²)], immediately after the impression was prepared, after seven days, after chemical treatment and during fluorescence examination if required.

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Table 1
Peroxidase reagents utilised in the study.

Chemical name	Alternative chemical name	Chemical supplier
Leucocrystal violet (LCV)	4,4′,4″-Methylidynetris(*N*,*N*-dimethylaniline)	Sigma Aldrich
Leucomalachite Green (LMG)	4,4′-Benzylidenebis(*N*,*N*-dimethylaniline)	Sigma Aldrich
Fluorescein	Acid Yellow 73	Sigma Aldrich
Luminol	3-Aminophthalhydrazide	Bluestar Forensic Magnum

Table 2
Fabrics utilised in the study.

Fabric	Supplier
White cotton [CD13] Plain weave; 19 warp threads/cm; 10 weft threads/cm	WBL Whaleys Bradford Ltd.
Black cotton [CD13D] Plain weave; 19 warp threads/cm; 10 weft threads/cm	WBL Whaleys Bradford Ltd.
Patterned cotton [SF2360/B] Twill weave; 19 warp threads/cm; 19 weft threads/cm	WBL Whaleys Bradford Ltd.
White polyester taffeta [SF25]	WBL Whaleys Bradford Ltd.
Black polyester taffeta [SF25A]	WBL Whaleys Bradford Ltd.
White nylon (82%)/lycra (18%) [SF28]	WBL Whaleys Bradford Ltd.
Black nylon (82%)/lycra (18%) [SF27]	WBL Whaleys Bradford Ltd.
Blue denim [rialto indigo] Twill weave; 25 warp threads/cm; 19 weft threads/cm	Mandors, Glasgow, UK
Brown bovine leather	The Clyde Leather Co., Glasgow, UK

2.1.1. Fluorescence photography

Fluorescence photography was operated as described in Part 1 of this study [43].

2.1.2. Computer monitor and colour calibration

Computer monitor and colour calibration was achieved as described in Part 1 of this study [43].

2.2. Peroxidase reagent formulations

The peroxidase reagents and fabrics utilised in the study are listed in Tables 1 and 2.

2.2.1. LCV formulation

LCV was prepared using the formulation suggested by Bodziak [13] incorporating the fix 5-sulfosalicylic acid dihydrate for a one-step process. 10 g of 5-sulfosalicylic acid (Acros) was dissolved in 500 mL of 3% hydrogen peroxide (VWR). 3.7 g sodium acetate (Sigma) was added to the mixture followed by 1 g of leuco crystal violet (Sigma) and stirred using a magnetic stirrer until completely dissolved. The reagent was applied by spraying with an Ecospray® unit supplied by Bluestar® Forensic. Fluorescence observation was carried out with a Mason Vactron Quaser 40 high intensity light source using a green/yellow excitation source (503–591 nm) and a red viewing filter (593 nm). For comparison purposed, a yellow laser (577 nm) was also employed for fluorescence examination and viewed with a red viewing filter.

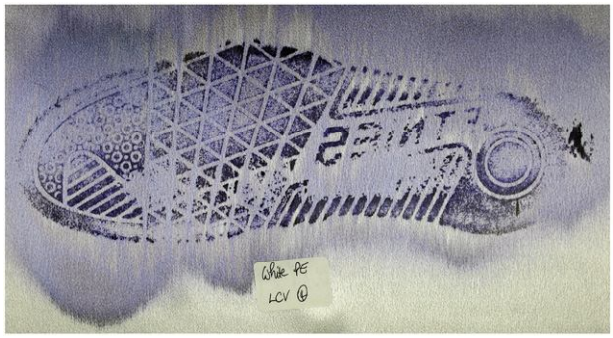

Fig. 3. LCV background staining on white polyester within 30 min of application.

2.2.2. LMG formulation

The LMG formulation utilised in this study was prepared as suggested by the Royal Canadian Mounted Police (RCMP) [44]. 0.2 g of leucomalachite green (BDH) was dissolved in 67 mL of methanol (Sigma) using a clean, dry, glass beaker. To this was added 33 mL of glacial acetic acid (Sigma) and 0.67 g of sodium perborate (Sigma) and the solution stirred (Sigma) using a magnetic stirrer until the LMG had completely dissolved. 300 mL of HFE 7100 (3M Novec) was finally added and the solution stirred. The resulting solution was stored in a dark coloured glass bottle and was applied by spraying with a Preval® sprayer.

2.2.3. Fluorescein

The fluorescein formulation was prepared according to Cheeseman [18–20].

Solution A: a 10% NaOH solution was prepared by dissolving 10 g of NaOH (Sigma) in 100 mL of distilled water. 1 g of fluorescein (Sigma) was dissolved in 100 mL of the 10% NaOH solution. The fluorescein solution was stirred and heated gently before adding 10 g of zinc powder (BDH) and the solution brought to a gentle boil before being left to cool. The cooled solution was then decanted carefully to remove the zinc which was neutralised prior to disposal. A 1:20 ratio of this solution with distilled water was then prepared.

Solution B: a 10% H_2O_2 solution was prepared by using 100 mL 30% H_2O_2 (VWR) and 200 mL distilled water.

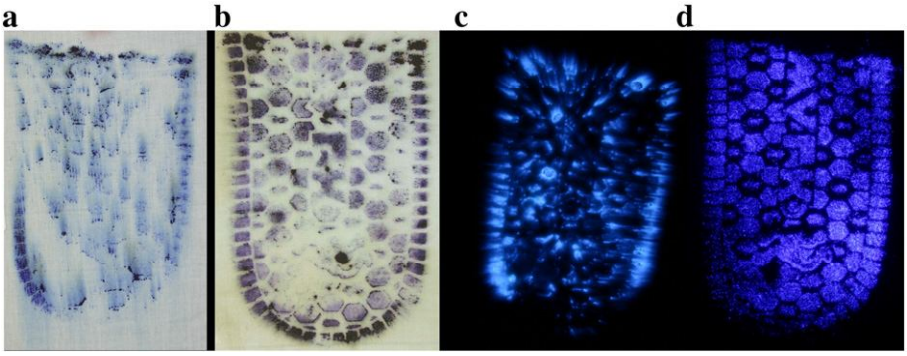

Fig. 2. Enhancement of footwear impressions in blood on white cotton using a BVDA sprayer for application of (a) LCV and (c) luminol — and Ecospray® for application of (b) LCV and (d) luminol.

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Fig. 4. Enhancement of a footwear impression in blood on patterned cotton using LCV: (a) before; (b) after.

The reagents were applied by spraying solution A followed by solution B using an Ecospray® unit supplied by Bluestar® Forensic. Fluorescence observation was carried out with a Mason Vactron Quaser 40 high intensity light source using an excitation waveband of 385–509 nm and a viewing filter of 510 nm.

2.2.4. Luminol

The luminol formulation utilised in this study was Bluestar® Forensic Magnum purchased from Bluestar® Forensic. It was prepared by dissolving the three tablets in 125 mL of the liquid supplied and the reagent was applied using an Ecospray® unit supplied by Bluestar® Forensic. The best photographic quality of the resultant chemiluminescent reactions was obtained using a Canon EOS 300D digital camera set at ISO400, f 5.6, exposure of 15 s and white balance set on tungsten, as recommended by the Home Office Scientific Development Branch (HOSDB) [45].

2.3. Diminishing series

A diminishing series was prepared by stepping on a blood soaked tissue and then using the footwear rig to produce ten impressions in blood for each fabric with the first one being the most blood-stained. In this case the excess blood was not removed after the initial application. After seven days, the impressions were cut into four pieces and one part was treated with acid yellow 7 (AY7), a protein stain to provide a comparison for the peroxidase reagents, and the other three parts treated with LCV, LMG and luminol

2.4. Washing

The blood impressions were prepared as detailed previously and left for 48 hours before washing in a Hoover® washing machine with Surf® powder detergent at a temperature of 40 °C on a general cycle for medium-soiled laundry. The samples were left to dry overnight before chemical treatment using AY7, LCV, LMG and luminol.

3. Results and discussion

A number of methods are reported for the application of the various peroxidase reagents onto the receiving surface. Sprayers available from Preval®, BVDA (Netherlands), Bluestar® and a conventional garden sprayer were all evaluated as a means of reagent delivery for LCV, LMG and Luminol.

Diffusion of the original impressions was observed in almost all cases after application of the reagents using either a garden sprayer or

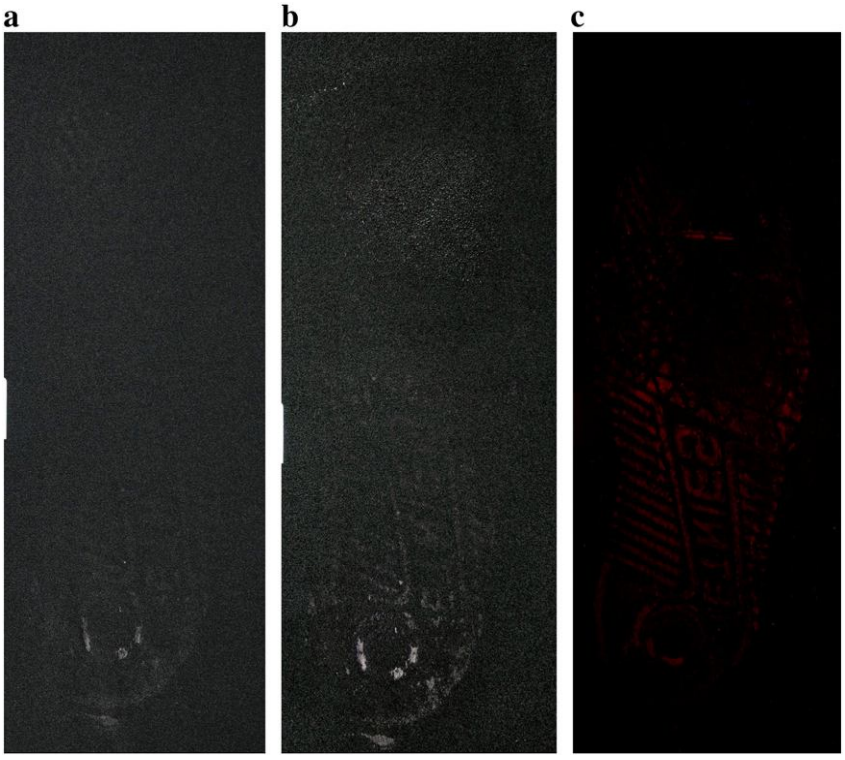

Fig. 5. Enhancement of a footwear impression in blood on black nylon/lycra using LCV: (a) before enhancement; (b) under white light; (c) using fluorescence.

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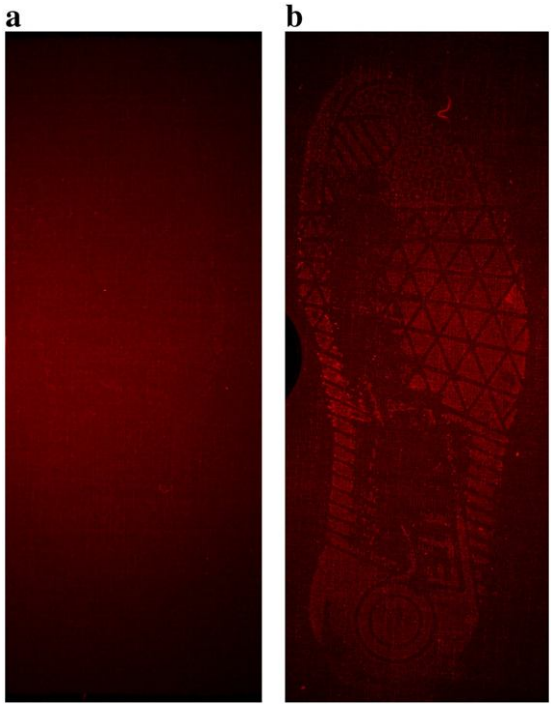

Fig. 6. Enhancement of a footwear impression in blood on denim and enhanced with LCV using a (a) Quaser 40 yellow/green excitation source (503–591 nm) and (b) yellow laser (577 nm).

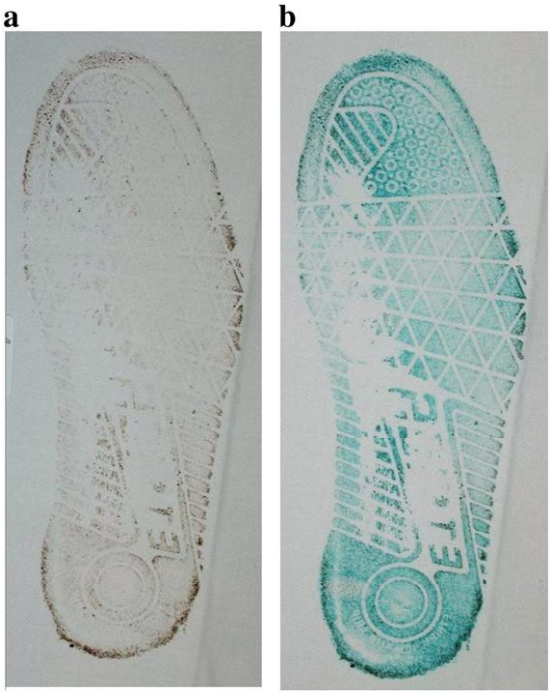

Fig. 8. Enhancement of a footwear impression in blood on white cotton using LMG: (a) before; (b) after.

a BVDA sprayer. After several attempts, the Bluestar® Ecospray unit proved to be the only spray suitable for enhancing impressions in blood without diffusion and obliteration of the original impression for luminol and LCV. This is in line with recent research at HOSDB where the Bluestar® Ecospray unit was found to be one of the best sprayers for the application of luminol in order to avoid diffusion of the impression in blood [46]. The Ecospray® unit delivered a very fine mist and even repetitive light applications of luminol on the same impressions failed to cause diffusion. An example of the result obtained for LCV and luminol is illustrated in Fig. 2.

For LMG, the Preval® sprayer provided better enhancement than the Ecospray® unit. This is most likely due to the fact that the fine mist of LMG produced by the Ecospray® unit was not sufficient to produce a vivid colour reaction with blood. It is also postulated that the fine mist delivery of HFE-7100 using Ecospray® evaporised quickly in the fume-hood to hinder the LMG enhancement.

Fig. 7. Enhancement of a footwear impression in blood on white cotton using LMG: (a) with fix; (b) without fix.

Fig. 9. Enhancement of a footwear impression in blood on denim using LMG: (a) before; (b) after.

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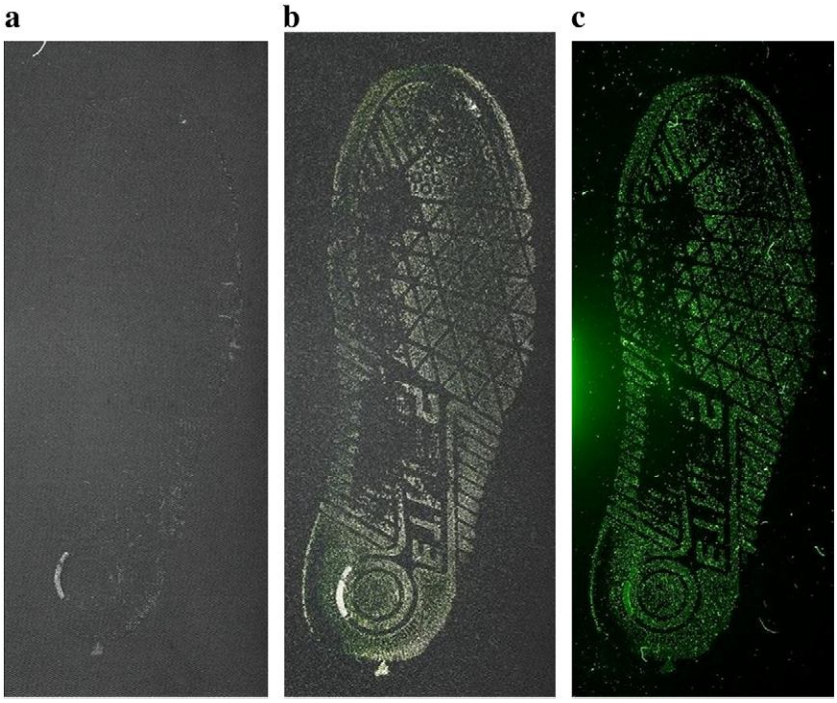

Fig. 10. Enhancement of a footwear impression in blood on black cotton using fluorescein: (a) before enhancement; (b) under white light; (c) using fluorescence.

3.1. LCV

The purple enhancement during LCV application is due to the haemoglobin catalysing the oxidation reaction of the colourless leuco crystal violet to crystal violet. A well-documented issue with this is that, when exposed to light, the oxidation reaction gradually causes the whole background surface upon which the target impression resides to turn purple [1,2,13,14]. An example of background staining is shown in Fig. 3. This can limit the operational use of LCV, however, rapid photography of the impression after the reagent application can offset this limitation. The ease of LCV application (via a spray without the requirement of fixing or de-staining) makes it an attractive enhancement technique for operational use.

LCV proved to be a suitable technique for the enhancement of blood impressions on all light coloured fabrics, including cotton (Fig. 4), polyester, nylon/lycra and leather, but failed to produce consistent enhancement for marks on denim and dark coloured fabrics. Contrary to the literature [13,15], examination of these fabrics using an alternate light source with different excitation filters failed to improve the visualisation of the enhanced marks produced. Examination of the LCV treated impressions with a Mason Vactron Quaser 40 green/yellow excitation source (503–591 nm) and viewed with a 593 nm viewing filter revealed weak fluorescence on black fabrics (Fig. 5). No fluorescence was observed for denim or leather substrates and fluorescence examination on light coloured fabrics did not improve on what could already be seen visually without fluorescence. The use of a yellow laser (577 nm) on black fabrics and leather did not improve the fluorescence observed using the Quaser 40, however, great improvement was obtained on denim as shown in Fig. 6. The human eye is relatively insensitive to dim red light and as a result, weakly fluorescing

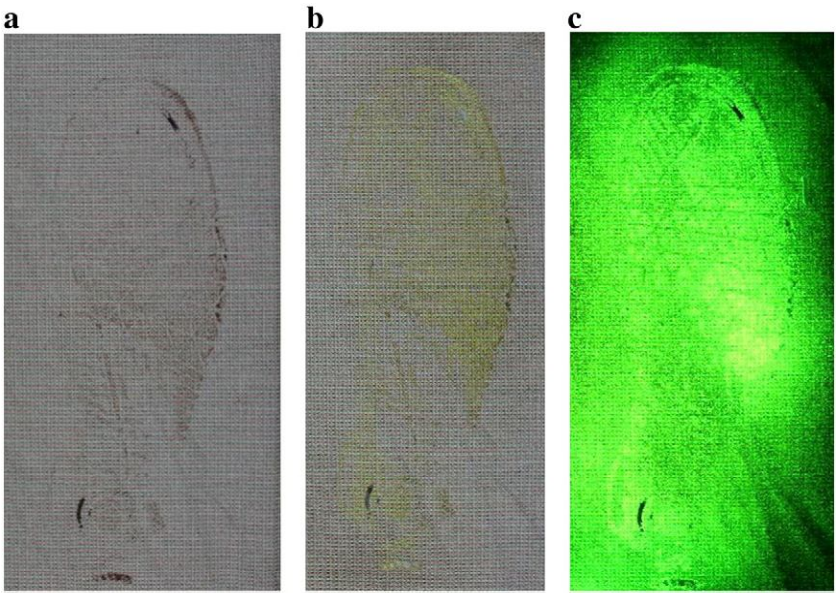

Fig. 11. Enhancement of a footwear impression in blood on patterned cotton using fluorescein: (a) before enhancement; (b) under white light; (c) using fluorescence.

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impressions may be missed unless the eyes are dark adapted for a period of about 30 minutes prior to observation [47]. The application of oblique lighting (Crime-Lite® 80 L) on black polyester did help visualise the enhanced impression slightly. Other problems associated with the use of LCV concern health and safety as crystal violet (the product formed from the reaction of blood and LCV) has recently been upgraded to a category 3 carcinogen [2]. Protein stains can also be applied after LCV.

3.2. LMG

The RCMP formulation [44,48] for LMG recommends pre-fixing the impressions with methanol. Trials carried out during this study showed that fixing the impression with methanol, ethanol or 5-sulfosalicylic acid resulted in a blurred enhanced impression with less vivid green colours. Repeated spraying of the impression also resulted in blurred impressions and best enhancement was achieved by not fixing the impression prior to application of the reagent. This is illustrated in Fig. 7.

LMG enhancement of the footwear impressions in blood performed in a manner similar to LCV, however, background staining did not occur. Slight diffusion of the enhanced impression, minimised by lighter spraying, occurred on white polyester. Enhancement on light coloured fabrics was clear and sharp with a vivid green colour being observed as illustrated in Fig. 8. This is in contrast to previous research [2] where the use of LMG was not recommended as the enhancement achieved was described as poor with a pale product colour causing problems with background contrast.

Enhancement on dark coloured fabrics including denim and leather was not visible or was poor due to contrast problems. LMG enhancement on black cotton and nylon/lycra seemed to be bright at first glance but disappeared rapidly. This observation was more pronounced for denim (Fig. 9) where the green colour faded within approximately 30 seconds post application of the reagent. Repeated application of LMG failed to re-enhance the impression.

3.3. Fluorescein

Fluorescein enhancement was poor on light coloured fabrics and excellent on dark coloured fabrics. The colorimetric reaction was visible almost instantaneously on the dark fabrics and leather (Fig. 10), eliminating the requirement for an alternate light source. The visual enhancement on denim was poor, though fluorescence did aid visualisation of the impression on this fabric. Quick capture of the enhanced marks using photography was essential as the bright yellow colour produced on application of the reagents began to fade after a few minutes. Background staining was observed on white cotton, polyester and nylon/lycra as well as patterned cotton which interfered with the fluorescence as illustrated in Fig. 11. A tinge of pink also developed with the background staining on the white synthetic fabrics. Contrary to Budowle [22], no fluorescein background staining on denim was observed, either initially or over time (Fig. 12). On dark coloured fabrics the use of a Quaser 40 to observe fluorescein fluorescence provided optimal contrast for visualisation of the footwear impression.

The application of protein stains can also be performed after fluorescein if further enhancement is necessary.

Fig. 13. Enhancement of a footwear impression in blood on denim using luminol: (a) before; (b) after.

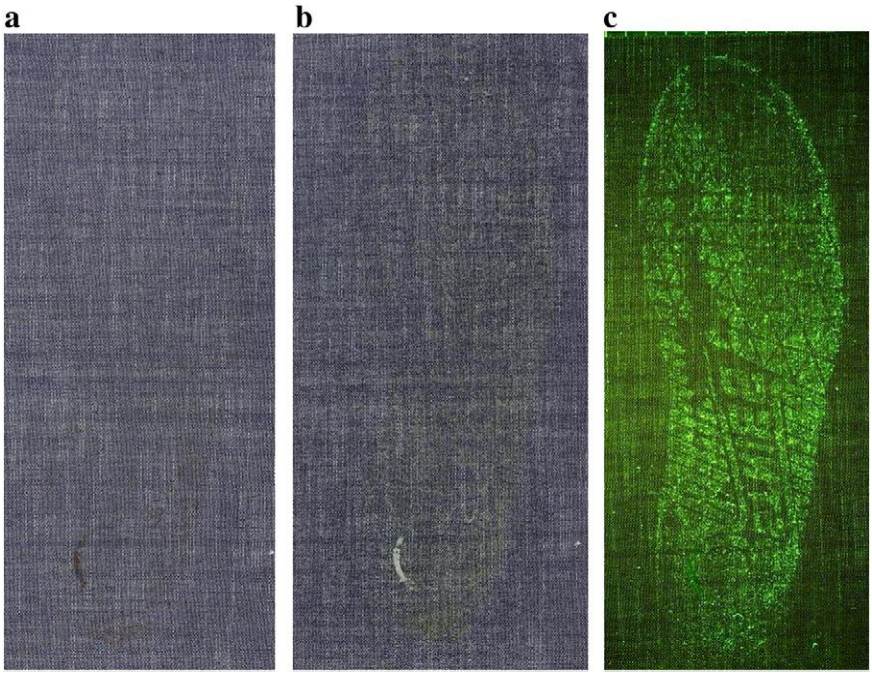

Fig. 12. Enhancement of a footwear impression in blood on denim using fluorescein: (a) before enhancement; (b) under white light; (c) using fluorescence.

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3.4. Luminol

Luminol proved to be the only technique successful in the enhancement of footwear impressions made in blood irrespective of fabric type or colour used in this study. The strongest enhancement on denim was achieved using luminol as illustrated in Fig. 13. Some diffusion and blurring was observed on the synthetic fabrics polyester and nylon/lycra (Fig. 14), in particular polyester. Several studies [19,21,23,38] have compared luminol and fluorescein with mixed views on which technique is the most efficient. Each have their advantages and disadvantages, however, in this study luminol performed better overall with fluorescein providing slightly less diffusion on black synthetic fabrics. No background staining was observed with the application of luminol on leather samples even though the reagent is reported to react with chromium and cobalt [49–51] often used in the tanning process of leather.

Luminol can be used before protein staining and other heme-reacting dyes. In general, the original impression remained visually unaffected by luminol and there was no permanent colouration. The application of luminol on the synthetic fabrics polyester and nylon/lycra exhibited slight diffusion, however, lighter spraying helped minimise this effect.

3.4.1. Diminishing series

The impressions from the diminishing series were cut into four pieces and treated with LCV, LMG, luminol and one protein stain (acid yellow 7) to provide an alternative fluorescence source by way of contrast. Fig. 15 shows an example of four pieces from a blood impression treated with the different enhancement techniques.

3.5. LMG and LCV

As previously discussed, both LMG and LCV enhancement on dark coloured fabrics did not provide good contrast. Nonetheless, the first two blood impressions prepared on dark fabrics were enhanced, presumably due to heavier blood staining. No marks on black polyester were enhanced by either reagent regardless of the quantity of blood imparted onto the fabric. Blood impressions on the light coloured fabric were enhanced with LMG and LCV up to the fourth or fifth impression. Diffused enhancement was observed on white polyester for both of these reagents.

Fig. 14. Enhancement of a footwear impression in blood on black nylon/lycra using luminol: (a) before; (b) after.

3.6. Luminol

Luminol has been widely reported for its sensitivity in the enhancement of blood stains [2,8] and it was hypothesised that luminol enhancement of the diminishing footwear impression series would be successful beyond the fifth impression. However the results demonstrated that, although luminol detected blood up to the tenth impression on most fabrics, the entire footwear sole could only be visualised up to the third or fourth impression at most. These results suggest that the footwear sole loses a lot of the accumulated blood after the first few impressions.

The results obtained for the full diminishing series for LCV, LMG and luminol for certain fabrics are illustrated in Figs. 16 to 18.

3.7. Acid yellow 7

AY7 provided better development of the blood impressions on black fabrics rather than light coloured fabrics and struggled to enhance past the first impression on denim and leather [43].

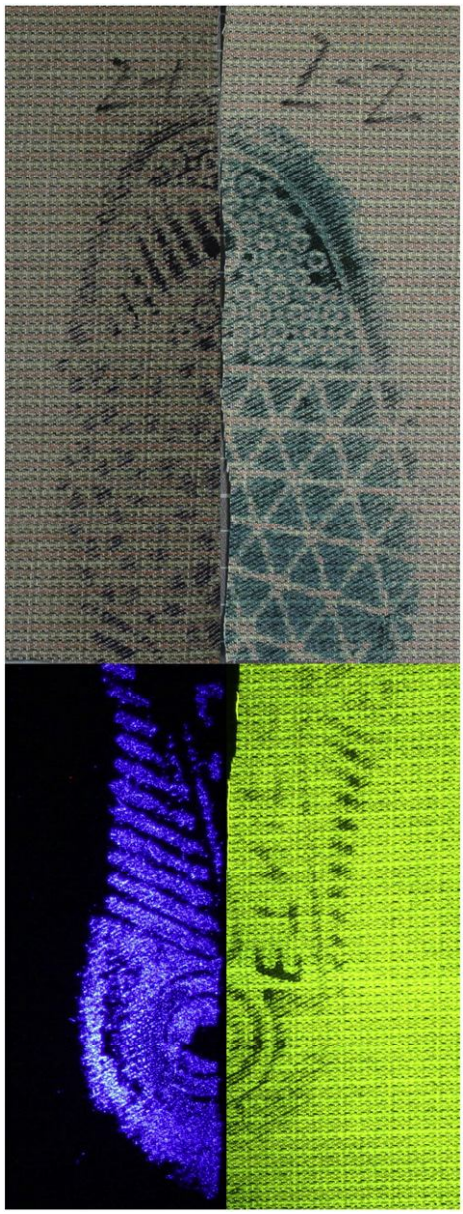

Fig. 15. The second impression of a diminishing series on patterned cotton treated with 4 different techniques (from top left going clockwise: LCV, LMG, acid yellow 7 and luminol.

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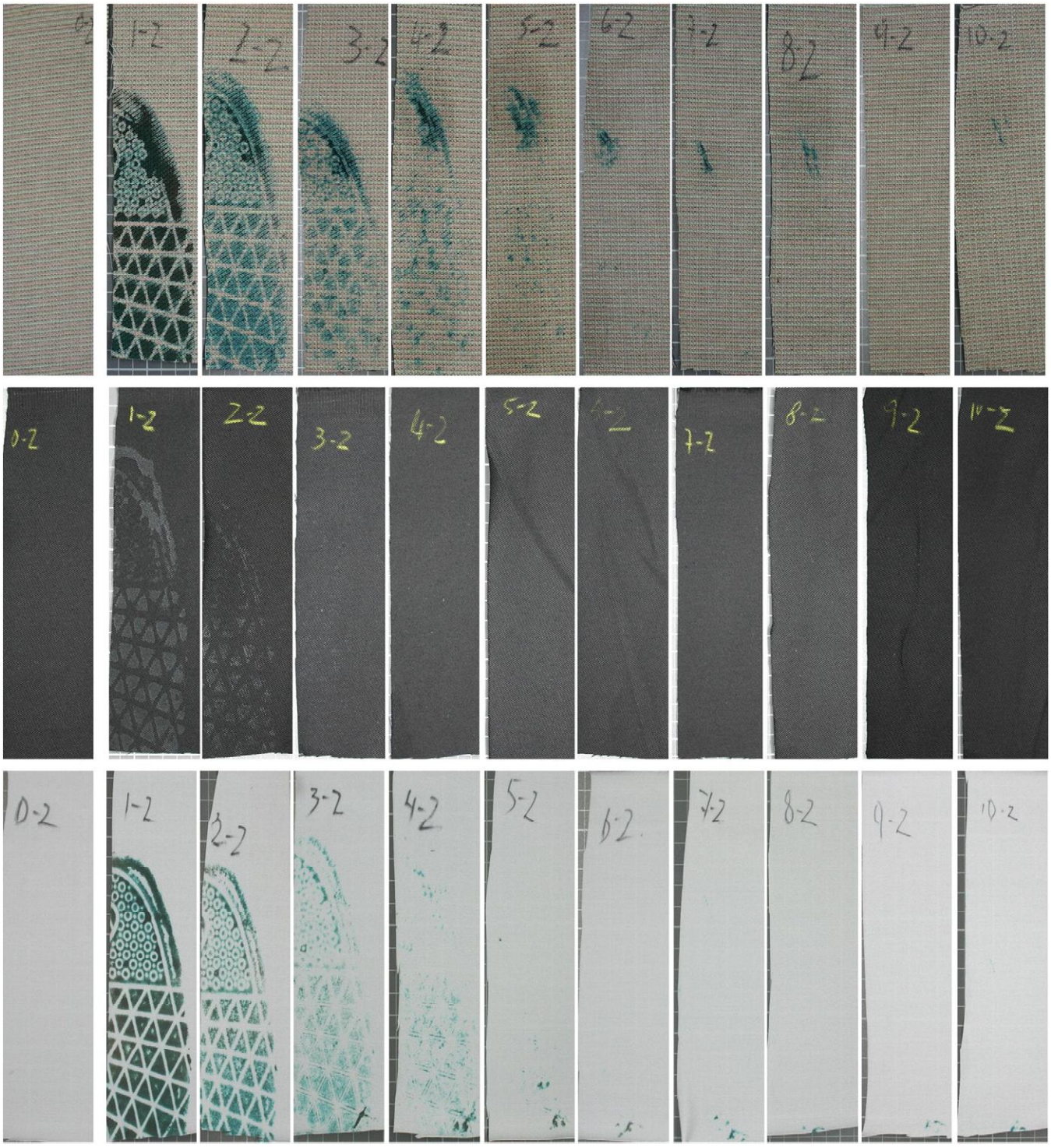

Fig. 16. LMG enhancement for a diminishing series in blood for: patterned cotton, black cotton and white nylon/lycra.

3.7.1. Washing

No enhancement of the footwear impressions was observed after washing and air drying for any of the reagents studied, although luminol showed slight scattered dots of chemiluminescence, indicating the potential presence of blood, however, this observation could also be due to drops of luminol and its inherent chemiluminescence. This is in contrast to previous research [52,53] where blood was detected using luminol and other techniques after washing. This difference can possibly be explained by the fact that in this study, weak latent blood impressions were prepared whereas in other studies heavier bloodstained impressions were used. Cox [53] also observed a relationship between the type of fabric and the retention of the bloodstains where blood was likely to wash off synthetic fabrics such as acetate, nylon and polyester.

4. Conclusion

The aim of this work was to provide a comprehensive comparison of the ability of a number of peroxidase reagents to enhance footwear impressions made in blood on a range of fabric types and colours. The

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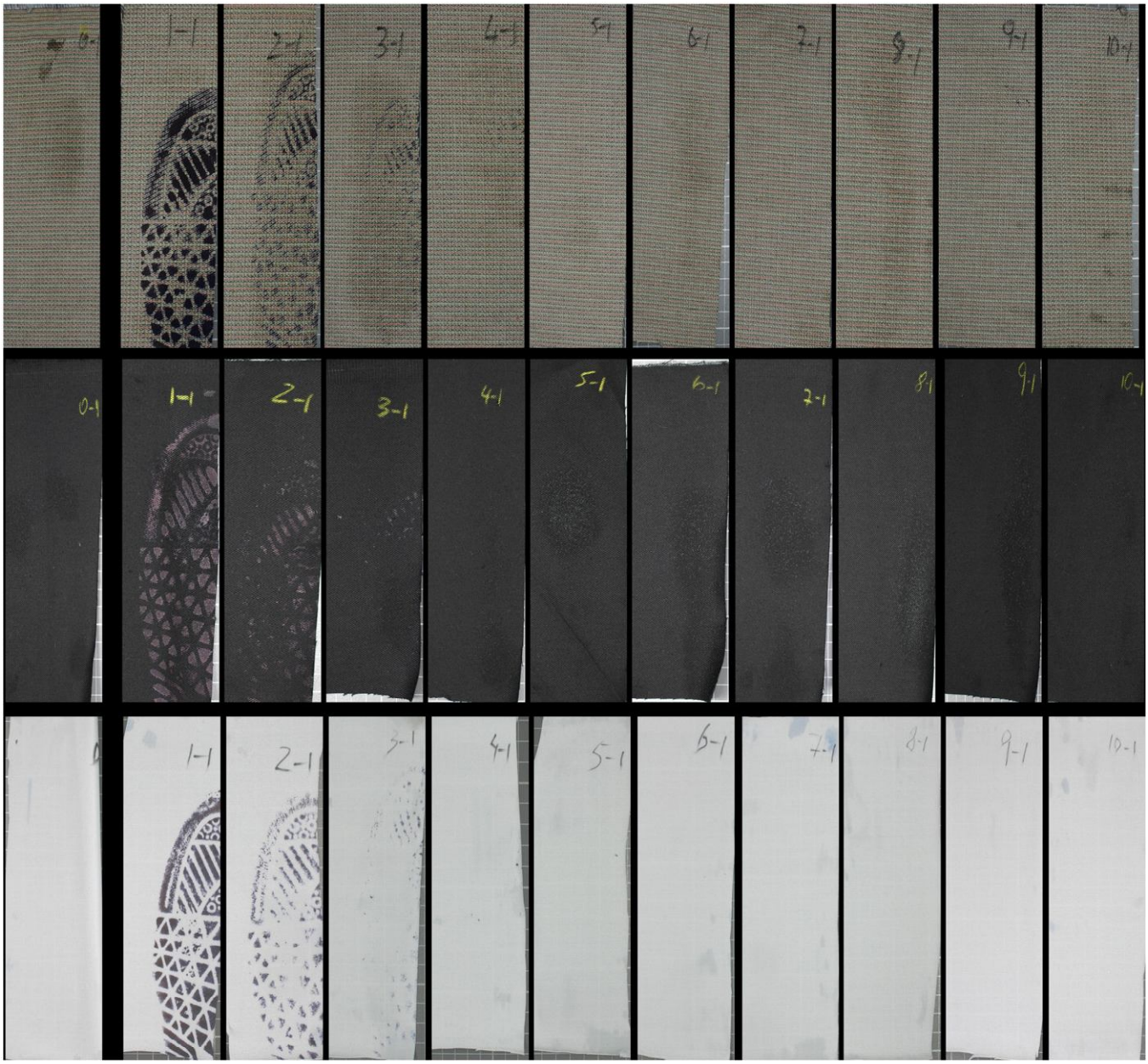

Fig. 17. LCV enhancement for a diminishing series in blood for: patterned cotton, black cotton and white nylon/lycra.

experimental methodology was chosen deliberately to control the means by which the impressions were produced and the quantity of blood used in each case so that the enhancement ability of the reagent was the only uncontrolled variable (within each fabric type), thus facilitating a true comparison between the reagents.

Of the four peroxidase reagents studied, Bluestar® Forensic Magnum luminol was the best performing enhancement technique overall, enhancing impressions on all surfaces and was the only technique to provide a clear enhancement of the impressions on denim. LCV and LMG provided good enhancement on patterned cotton and light coloured fabrics but were poor enhancers of impressions on darker fabrics whereas fluorescein and luminol provided excellent enhancement results due to optimal contrast with the background.

None of the peroxidase reagents successfully enhanced impressions in blood that had been subjected to washing.

In general luminol was the most efficient reagent for weaker impressions and provided good footwear detail up to the fifth impressed mark in a diminishing series. Similarly luminol detected blood up to the tenth impression for most fabrics where as the other reagents tested provided little enhancement past the second or third impression in the series.

Luminol provided excellent results on denim and leather where all other techniques performed poorly. However, acid yellow 7 and fluorescein appear to offer better results and less diffusion than luminol for the enhancement of footwear impressions in blood on black cotton, polyester and nylon/lycra.

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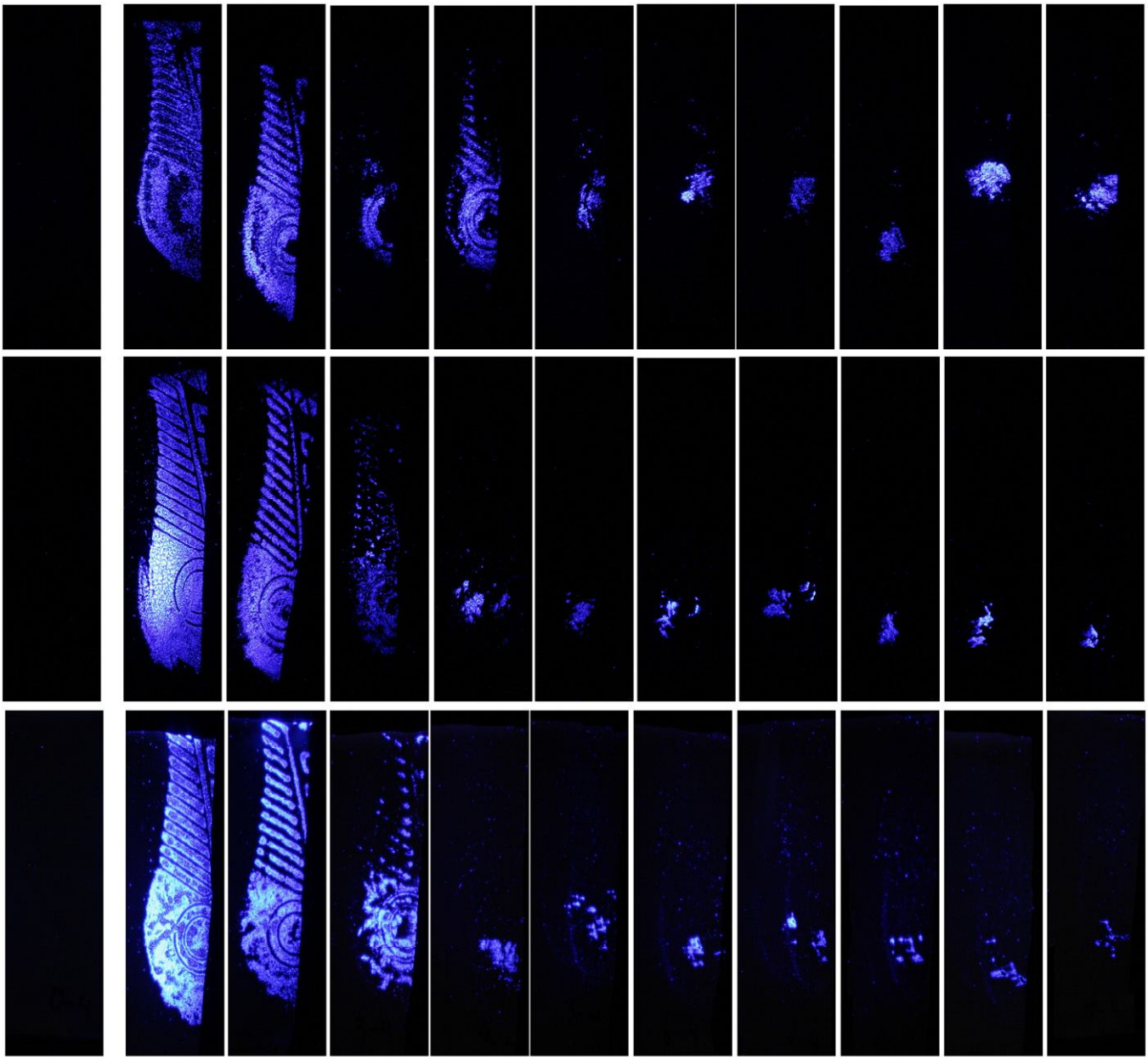

Fig. 18. Luminol enhancement for a diminishing series in blood for: patterned cotton, black cotton and white nylon/lycra.

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