

Article

Chemical Enhancement of Bloody Footwear Impressions from Buried Substrates

Siobhan Cullen
Alexandra Otto
Paul N. Cheetham

*Centre for Forensic Science,
School of Conservation Sciences
Bournemouth University
Poole, Dorset, UK*

Abstract: Footwear impressions are regarded as one of the most common forensic evidence types left at crime scenes. A review of research to date describes previous tests on the survival of footwear impressions in a range of contaminants on a myriad of surfaces. None, however, examined the effects of the burial environment on such impressions.

Using human blood as a contaminant, footwear impressions were made on samples of white cotton, newspaper, and black plastic trash bags and were buried for specific time frames, from one to four weeks. The study examines the subsequent development of the surviving impressions postexcavation, using chemical enhancement techniques of ninhydrin, acid black 1, leucocrystal violet (LCV), and Bluestar. The majority of impressions recovered were from the substrates that were in the soil for the shortest period. Poor recovery rates and loss of impressions were observed on substrates buried for more than two weeks. LCV and Bluestar proved most effective for enhancing and retrieving impressions. Impressions were able to be examined by a trained forensic footwear investigator to identify class, individual, and wear characteristics of the impression itself. Potential survival of such identifying features is of paramount importance to an investigation.

Received January 28, 2009; accepted May 15, 2009

Journal of Forensic Identification
60 (1), 2010 \ 45

Introduction

Footwear impression evidence is one of the most common forensic evidence types left at crime scenes and, if recovered and treated effectively, can provide substantial information relating to potential suspects. Every forensic archaeological investigation is unique and may include not only the recovery of human remains but also of related artifacts and property. The potential survival of footwear impressions on buried property therefore needs to be addressed and appropriate enhancement techniques employed.

Previous studies and experiments have examined the visualization and enhancement of forensic impressions within a plethora of situations and contaminants. Lytle and Hedgecock [1] examined the chemiluminescence in the visualization of forensic bloodstains. Doherty and Mooney [2] explored a series of chemical reagents with the intent to enhance bloody imprints to a legible degree. Bodziak has been a leader in the field in forensic footwear recovery, outlining the most effective detections, recovery, and examination procedures available [3, 4] and specifically looked at the application of leucocrystal violet (LCV) for the enhancement of shoe prints in blood [5]. Theeuwens et al. [6] studied various methods for the chemical enhancement of footwear impressions in blood. Ashe et al. [7] used fingerprint enhancing techniques (cyanoacrylate fuming followed by staining with panacryl brilliant flavin) to visualize latent footwear marks in grease or oil deposited on plastic bags. Sears et al. [8-10] examined reactive techniques and process sequences for the enhancement of fingermarks in blood. The Scientific Working Group on Shoeprint and Tire Tread Evidence (SWGTTREAD) outlined several guidelines for the detection, recovery, collection, examination, documentation, preparation, and enhancement of bloody impression evidence, both in the field and in the lab [11-19].

This study attempts to ascertain whether impressions will survive a test of time in relation to various materials found in the burial environment. It also addresses the length of time blood survives on these materials. Successful retrieval of this type of physical evidence would significantly help investigators to solve cases and provide a benchmark for the type of results that a forensic archaeologist or crime scene investigator may encounter at a forensic archaeological site or site where

property has been buried in relation to a crime. The findings of this study may prompt further research into techniques of excavation, recovery, preservation, and potential enhancement of buried bloodstained evidence.

Materials and Methods

The abundant range of materials that could be present at a crime scene, as well as the potential number of contaminants that may be deposited on these materials, go beyond the scope of this experiment. In this regard, blood was chosen as a common contaminant likely to be found on the materials selected for this experiment.

Methods Employed

The use of chemicals to investigate crime scenes is well known, and many methods and materials will successfully develop or enhance bloody footwear impressions. Two main categories of chemicals exist for this purpose: those that react to the amino acids and proteins contained in blood or other biological fluids and those that detect an enzymatic activity and involve catalytic, hemoglobin test reagents. Hemoglobin-specific reagents are often used in preference to protein stains because of their ease of application, which involves spraying a solution and observing a color change in the presence of blood. Although easier to apply, the enhanced blood marks may be weaker and more diffuse than if developed with a protein stain, and photography may be difficult [20]. The selection of which particular method to use is important and depends on several factors [3, 6, 8, 9, 10]:

- Nature of the blood impression
- Background colors of the substrate
- Nature of substrate
- Texture and porosity of the substrate
- Safety of the reagent
- Ease of preparing solutions
- Ease of applications
- Availability of equipment
- Expertise of the examiner

The following chemicals were chosen for this experiment: ninhydrin, acid black 1, LCV, and Bluestar. The former two react to the amino acids and proteins in the blood, whereas the latter two are catalytic hemoglobin-specific tests. Features of each chemical and the reagent preparation procedure are outlined below.

Ninhydrin

Ninhydrin is an amino acid-developing reagent that is applied by dipping, brushing, or spraying. Development is catalyzed by the addition of heat, which will accelerate the reaction. A positive reaction will produce a dark color called Ruhemann's purple. It is a very effective reagent for porous surfaces [6, 21-23].

A special formula spray solution (Sirchie, #NSI609) containing ninhydrin, ethylacetate, and 1-methoxynonafluorobutane (a volatile solvent that displaces air above the solution) was used. Application was via a spray bottle.

Acid Black 1

Acid black 1 is a protein stain that produces a dark blue-black color in areas where blood is present. It is a dye-staining process, followed by rinsing, that can be used to enhance detail on faint bloody impressions both on porous and nonporous surfaces [6, 8, 21-24].

Aqueous acid black solution (Lightning Powder Company #1-2740) was used. Application was via immersion and was applied in a fume hood.

Leucocrystal Violet

LCV is a simple, safe, and effective reagent for blood enhancement and development on both porous and nonporous surfaces. Spraying is the most effective means of application [21]. When LCV and hydrogen peroxide come into contact with the hemoglobin in blood, a catalytic reaction occurs and the product turns purple-violet. The application of LCV provides a quick and uncomplicated method of visualizing and enhancing impressions [5, 6, 21, 25-27].

Aqueous LCV (Sirchie #LV509) was used and consisted of three different compounds that had to be mixed prior to application:

Part 1: an aqueous solution containing hydrogen peroxide and 5-sulfosalicylic acid

Part 2: sodium acetate

Part 3: LCV

Parts 1 and 2 were combined and shaken. Part 3 was then added to the mixture and shaken for approximately three minutes. The reagent was then ready for use. Application was via a spray bottle.

Bluestar Forensic

Bluestar is a powerful bloodstain reagent that can be used on all surfaces. When mixed with the catalyst hydrogen peroxide and put into contact with the hemoglobin in blood, Bluestar oxidizes and omits an intense blue chemiluminescence, which is best seen if the product is applied in total darkness [28]. Young reaffirmed and quoted the manufacturer's claims that Bluestar's advantages include "stronger luminescence, longer lasting reaction, higher sensitivity, total darkness not required, photos shot with ordinary camera, fully soluble, stable over time, easy to use, and non toxic" [29]. Dilbeck also determined that Bluestar was "exceptionally better than luminol in the following areas: ease of mixing, lack of complete darkness, and good intensity after initial spray" [30].

A Bluestar Forensic kit (Bluestar Forensic, #BL-FOR-BLUEST) was used. It contained 500 mL (16 oz) of reagent, three catalyst tablets, and a fine mist atomizer. The three catalyst tablets were dissolved in the solution of the bottle. Application was via spraying.

Substrate Selection

The performance of the reagent depends a lot on the nature of the substrate. The porosity and composition of the materials will affect the reaction of the reagents, therefore, both porous and nonporous materials were chosen.

- A plain white 100% cotton T-shirt
- A newspaper
- A black polyethylene trash bag

The reason for choosing these substrates was that they are common items that have the potential to be found at scenes of crime or burial sites. Therefore, their potential degradation within the burial environment, and the survival of bloodmarks on both porous and nonporous items, could be examined.

Sixteen samples of each substrate were used. A total number of 48 samples were buried in the plastic containers to be tested over a period of four weeks by the chemicals previously mentioned.

Blood

Human blood was supplied by a blood bank. The blood had not been treated in any way, other than having white cells removed. It was stored in a laboratory refrigerator at 5.2 °C. The blood was poured onto a plastic tray on the days the experiment was being conducted.

Shoestamp

The authors wore an Adidas shoe (size 8), walked on the blood tray, and then made impressions on the various substrates. Only the heel part of the impression was used (Figure 1), because it contained several distinguishing characteristics for the purposes of the experiment. The dimensions of the heel of the shoe were approximately 11 cm x 9 cm.

Grading Samples

A depletion series of impressions (Figure 2) was made on white paper and was used as a standard grading system on which to base the grading of impressions made and retrieved from the substrates at preburial, postexcavation, and post-treatment stages. The twelve impressions were evaluated and grades of 0 to 3 inclusively were assigned, depending on the visibility of the detail (Table 1).



Figure 1

Shoestamp used for making impressions.

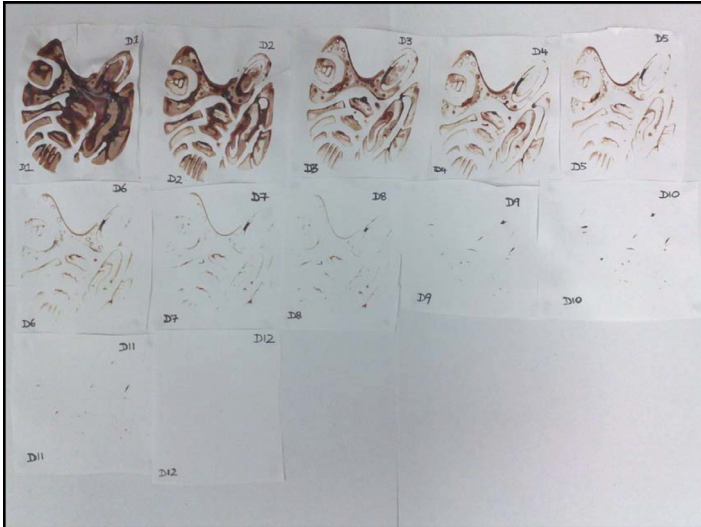


Figure 2

A depletion series of twelve impressions used as a standard for grading the samples.

Photography

Photographs were taken using a 5 megapixel digital single lens reflex camera and included a right angle scale.

Experiment Procedure

The experiment was conducted over a period of four weeks.

Number of Impressions

The substrates were taped to the ground so as to negate any potential destruction caused by movement of the materials, and the impressions were made by walking over the taped substrates. On a weekly basis, a set of twelve impressions were made (four on each substrate) and left to dry overnight (Table 2). The footstamp was reloaded for each impression made to obtain the best possible detail. Control samples were also prepared and left to dry.

Burial of Substrates

The twelve samples were buried on a weekly basis in plastic containers in the following manner: Sieved soil (5 cm) was put in each container. Samples 1 through 4 (of each substrate) were placed along one side of the container face down in the soil so as to maximize contact. Sieved soil (5 cm) was then poured on top of the samples from a height of approximately 2 cm. The following week samples 5 through 8 were placed along the other side of the containers, and 5 cm of soil was placed on top. The process was repeated to bury samples 9 through 16 on top (Figure 3). The containers were kept indoors with an average ambient temperature of 23 °C.

Four control impressions were also made of each substrate. These controls were not buried but were placed in a plastic box for the same periods of time as their counterparts.

Samples of substrates with no blood were also buried as controls on the same days. These would be tested at the conclusion of the experiment.

Gloves were worn at all times to prevent contamination and to decrease the risk of obtaining false positive reactions when testing because of the presence of latent fingerprints.

Depletion Number	Represents	Grade	Score
1, 2, 3	Mark with clear detail	Excellent	3
4, 5, 6	Partial detail in mark	Good	2
7, 8, 9	Smudged mark with no clear detail	Poor	1
10, 11, 12	No clear mark or detail	No value	0

Table 1

Grading system for diminishing series.

	T-shirt				Newspaper				Trash bag			
4 weeks	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
3 weeks	A5	A6	A7	A8	B5	B6	B7	B8	C5	C6	C7	C8
2 weeks	A9	A10	A11	A12	B9	B10	B11	B12	C9	C10	C11	C12
1 week	A13	A14	A15	A16	B13	B14	B15	B16	C13	C14	C15	C16

Table 2

Numbering of samples.

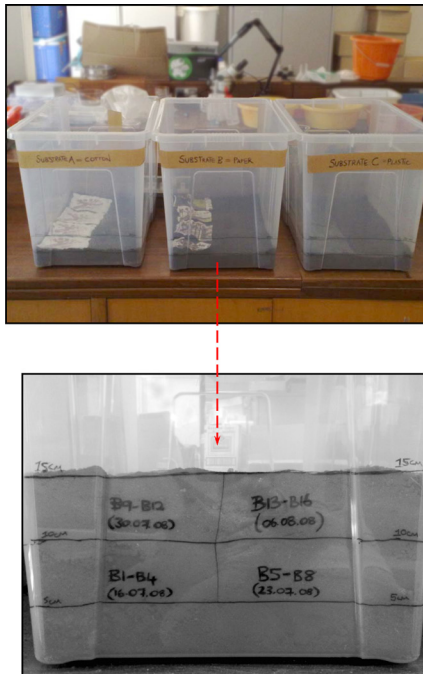


Figure 3

Burial of substrates.

Excavation of Substrates

The excavation method for the recovery of the samples was consistent throughout the experiment. When the location of each sample was revealed, the sample was simply lifted to prevent further abrasion [31].

Testing of Substrates

Extraneous soil particles were meticulously removed from each substrate using a small hand brush, and photographs were taken prior to testing.

	Ninhydrin	Acid black 1	LCV	Bluestar
In soil 4 weeks	A1	A2	A3	A4
In soil 3 weeks	A5	A6	A7	A8
In soil 2 weeks	A9	A10	A11	A12
In soil 1 week	A13	A14	A15	A16
In soil 4 weeks	B1	B2	B3	B4
In soil 3 weeks	B5	B6	B7	B8
In soil 2 weeks	B9	B10	B11	B12
In soil 1 week	B13	B14	B15	B16
In soil 4 weeks	C1	C2	C3	C4
In soil 3 weeks	C5	C6	C7	C8
In soil 2 weeks	C9	C10	C11	C12
In soil 1 week	C13	C14	C15	C16

Table 3

Substrates and enhancement methods applied to each.

Chemical enhancement methods were applied to the substrates (Table 3) in the following manner:

Application of Ninhydrin

A special formula ninhydrin spray that was quick drying and nonsmudging was used. The spray bottle was held four to six inches from the surface and the entire area was sprayed uniformly until damp. The substrates were left to dry for approximately one hour, after which time they were exposed to elevated heat to hasten the result. This was achieved by placing a steam iron at full heat for one to two minutes over the substrate.

Application of Acid Black 1

Acid black 1 aqueous solution was poured onto a plastic tray. The application to the substrates required the use of a fume cupboard. Substrates were immersed in the solution for a period of two to three minutes after which time they were removed and rinsed with distilled water. Substrates were left to dry for approximately two hours.

Application of LCV

LCV was applied to the substrates with a fine mist sprayer. After thirty seconds, the developed stains were lightly blotted with clean paper towels. After blotting, the surface was dry, and additional reagent was applied to weaker stains. All visible prints were photographed immediately.

Application of Bluestar

The application of Bluestar took place in the darkroom of the university. Results were achieved by spraying the substrates with the premixed solution from a height of four to six inches and photographing within ten seconds of application.

Results

Preburial Evaluations

Of the 48 original impressions, 29 were of excellent quality, 12 were good, and 7 were poor. All could be considered to have potential to be compared with a known impression from a suspect. Figure 4 shows the results of the preburial impressions.

Postexcavation Evaluations

All 48 impressions were buried in the methods previously outlined. Figure 5 shows the results of the postexcavation impressions.

Of the 48 samples of footwear impressions that were made with blood on the various substrates and buried, only 29 showed visible signs of impressions on excavation (60%). Of the 29 visible impressions recovered, 12 were of excellent quality, 6 were good, and 11 were poor.

The recovery rate varied between substrates over the length of time of the experiment:

- Four weeks in the soil totaled 1 visible impression (1 from A, 0 from B, 0 from C).
- Three weeks in the soil totaled 8 visible impressions (4 from A, 4 from B, 0 from C).
- Two weeks in the soil totaled 8 visible impressions (4 from A, 4 from B, 0 from C).
- One week in the soil totaled 12 visible impressions (4 from A, 4 from B, 4 from C).

Substrates buried for one week proved to have the highest recovery rate of visible impressions. These were in the soil for the shortest period of time, with only one impression being visible after four weeks in the soil (1 from A).

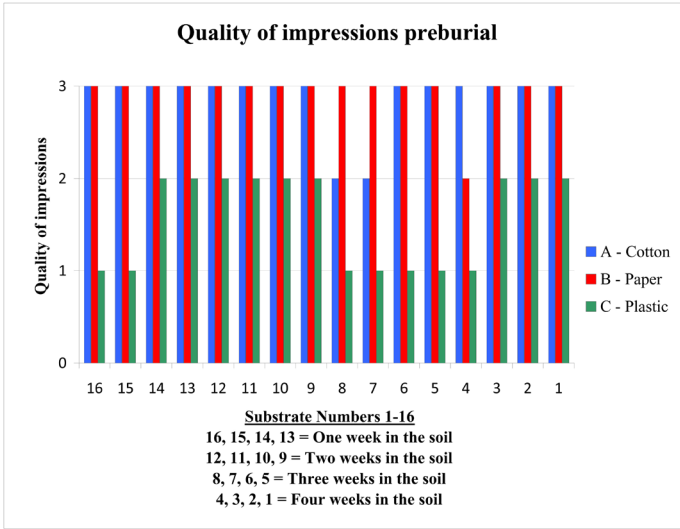


Figure 4

Quality of impressions preburial.

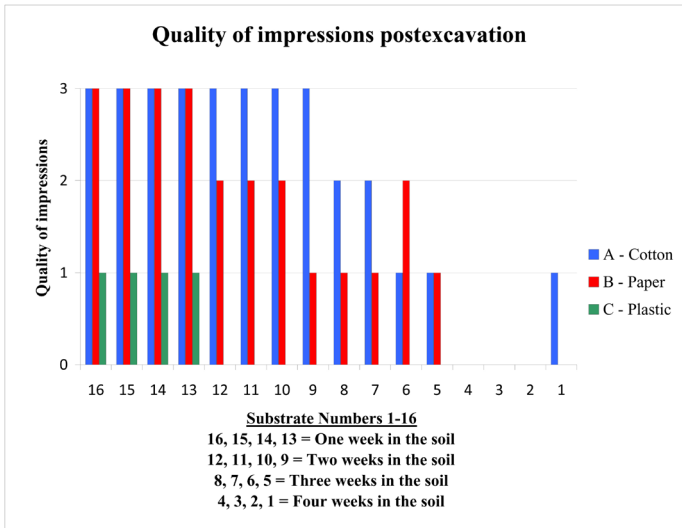


Figure 5

Quality of impressions postexcavation.

Post-treatment

Substrates were treated with the enhancement methods previously outlined. Results are shown in Figure 6.

Of the 48 samples buried and subsequently excavated (six samples shown in appendix A), only 35 provided adequate visible impressions following chemical enhancement (73%). Fourteen of these were of excellent quality, 10 were good, and 11 were poor. Ten gave no clear results whatsoever, and 3 were completely destroyed.

Treatment of all excavated substrates also resulted in differential recovery rates, regardless of the presence of visible impressions (or lack thereof):

- Four weeks in the soil totaled 8 recovered impressions (3 from A, 4 from B, 1 from C).
- Three weeks in the soil totaled 8 recovered impressions (3 from A, 4 from B, 1 from C).
- Two weeks in the soil totaled 9 recovered impressions (4 from A, 3 from B, 2 from C).
- One week in the soil totaled 10 recovered impressions (4 from A, 4 from B, 2 from C).

Again, most impressions were recovered from those substrates that had been in the soil for the shortest period (one week).

Review of Results Per Week

The following outlines the results observed on each of the substrates per week:

Cotton

Substrate numbers: A16, A15, A14, A13 (Figure 7)

All impressions were excellent on burial and postexcavation. Seventy-five percent of results post-treatment were also excellent. Bluestar gave only a good result, probably because of poor application or pooling of the solution.

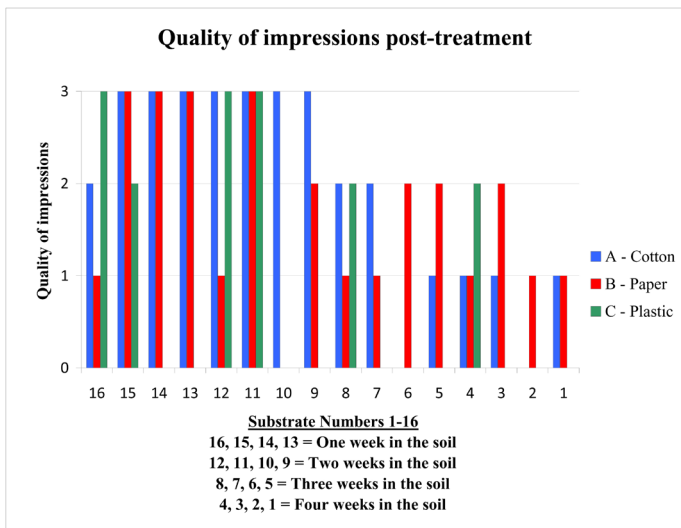


Figure 6

Quality of impressions post-treatment.

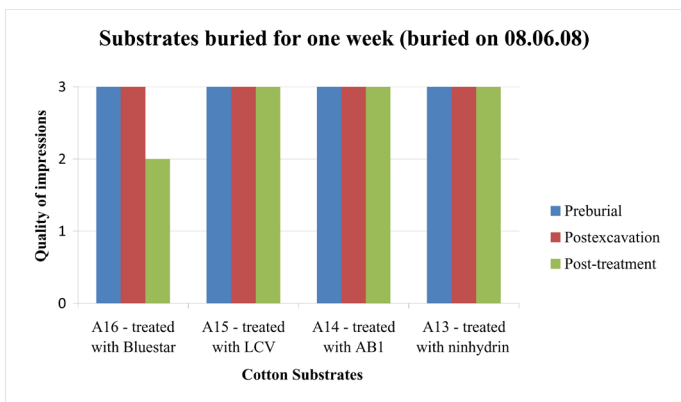


Figure 7

Comparison of cotton substrates buried for one week.

Substrate numbers: A12, A11, A10, A19 (Figure 8)

Results were almost exactly the same as those observed above. Initial impressions were excellent. Impressions on recovery from the soil after two weeks were excellent. Results of impressions post-treatment with all techniques were excellent.

Substrate numbers: A8, A7, A6, A5 (Figure 9)

A5 and A6 were of excellent quality, with A7 and A8 being good. Treatment of a poor impression recovered from A5 with ninhydrin did not improve it, however, it did not make it less recognizable. The only difference was a slight color change. After treatment of A7 and A8 with LCV and Bluestar, respectively, impressions were still good. A6, however, resulted in complete loss of material and impression when immersed in the acid black 1 solution. This shows the weakness of the material, which succumbed to complete disintegration when immersed in a water-based solution, after being in the soil for both four and three weeks.

These results show how much of a difference one week in the soil can make compared to results obtained from substrates buried for two weeks where no disintegration, no loss of impression, and ideal results were achieved as observed above.

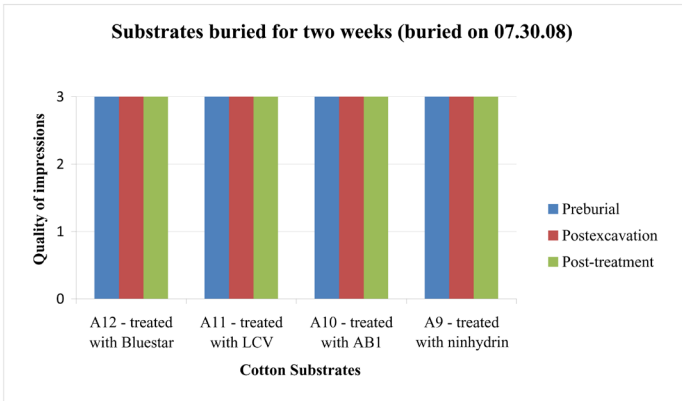


Figure 8

Comparison of cotton substrates buried for two weeks.

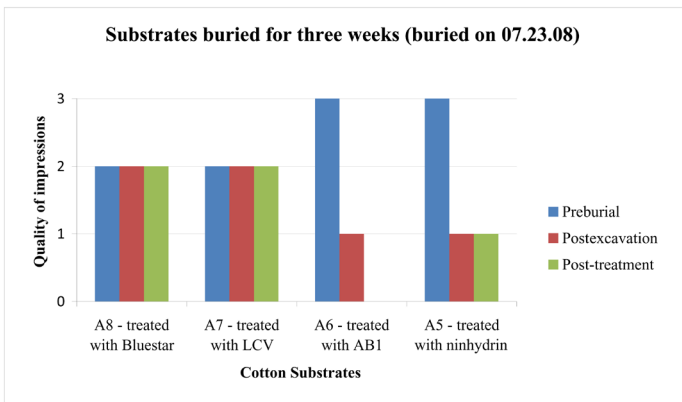


Figure 9

Comparison of cotton substrates buried for three weeks.

Substrate numbers: A4, A3, A2, A1 (Figure 10)

All initial impressions made on cotton were of excellent quality. After being in the soil for four weeks, however, the material disintegrated to a great extent, leaving any recognition of impressions almost impossible. Cleaning of the substrate postexcavation also proved very difficult because any further brushing rendered increased damage to the substrate. Treatment of these substrates with ninhydrin, LCV, and Bluestar gave poor results. However, immersion of the already deteriorated material in the acid black 1 solution resulted in complete loss of the substrate and, therefore, the impression.

On excavation, materials were more disintegrated, and no visible impressions were observed compared to one week previous.

Paper

Substrate numbers: B16, B15, B14, B13 (Figure 11)

After just one week in the soil, impressions recovered from paper were all excellent and, when treated with ninhydrin, acid black 1, and LCV, gave excellent results. Bluestar, however, only gave a poorly enhanced impression for comparison purposes.

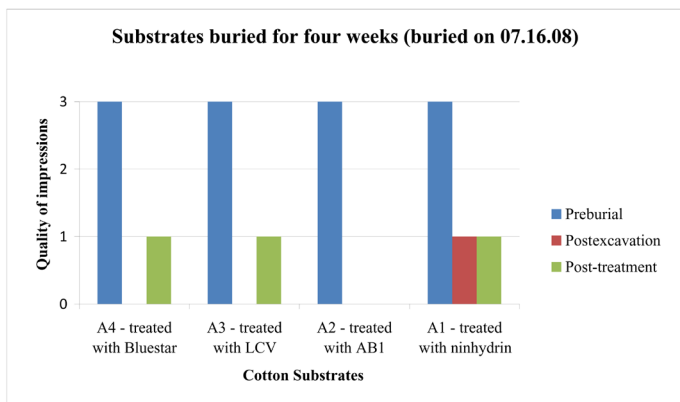


Figure 10

Comparison of cotton substrates buried for four weeks.

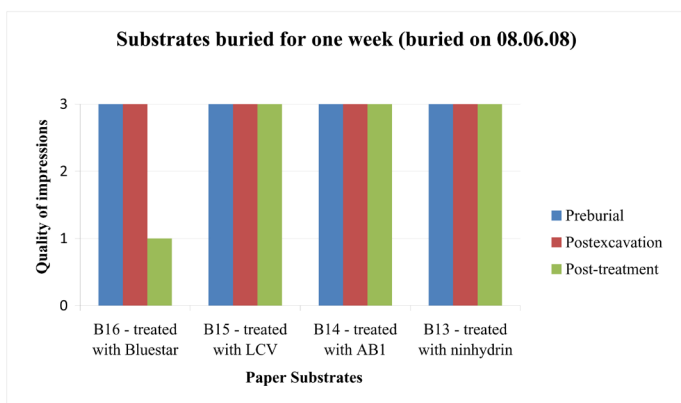


Figure 11

Comparison of paper substrates buried for one week.

Substrate numbers: B12, B11, B10, B9 (Figure 12)

Upon burial, impressions were all excellent. Those recovered after excavation were good and poor. Paper, therefore, does not retain the same quality of impressions as cotton, even though both were in the soil for two weeks, both had initial excellent impressions, and both were of a porous nature. Treatment of those recovered impressions showed an increase in quality with ninhydrin and LCV. However, acid black 1 again destroyed one sample (B10), and Bluestar only produced a poor result on this occasion.

Substrate numbers: B8, B7, B6, B5 (Figure 13)

Upon burial, all initial impressions were of excellent quality. Postexcavation, 75% of those recovered were poor; B6 was good. Treatment of B5 and B6 with ninhydrin and acid black 1, respectively, gave good results. LCV and Bluestar gave poor results after three weeks in the soil.

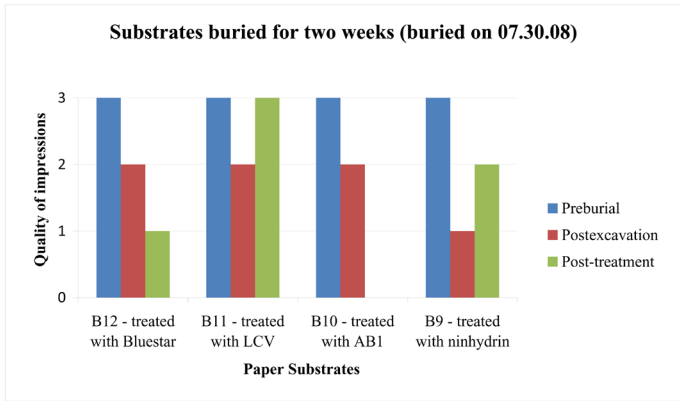


Figure 12

Comparison of paper substrates buried for two weeks.

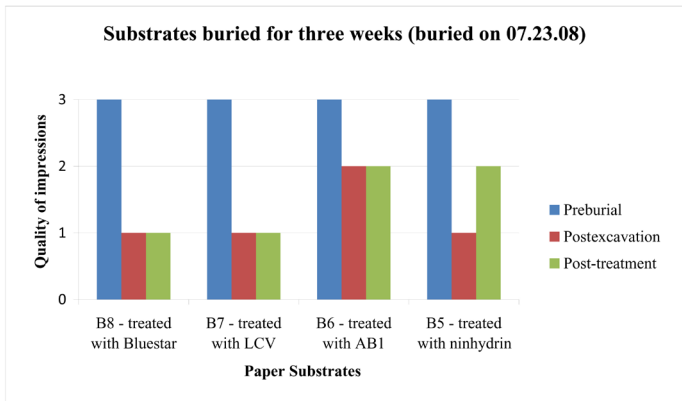


Figure 13

Comparison of paper substrates buried for three weeks.

Substrate numbers: B4, B3, B2, B1 (Figure 14)

B1, B2, and B3 impressions were excellent, and B4 was good. Again, after four weeks in the soil, disintegration of substrates occurred, most proving difficult to excavate because of the loss of material. The porous nature of the paper also increased the difficulty of excavation because it was more moist than other substrates. Treatment of B1, B2, and B4 with ninhydrin, acid black 1, and Bluestar, respectively, resulted in poor impressions. LCV gave a good response on this occasion.

Plastic

Substrate numbers: C16, C15, C14, C13 (Figure 15)

After only one week in the soil, samples showed good signs of recovery of impressions; those observed on excavation were of poor quality. Treatment of C13 with ninhydrin and C 14 with acid black 1 did not improve the result, and, therefore, the impression was lost. Treatment with LCV gave good results, and Bluestar gave an excellent enhanced impression.

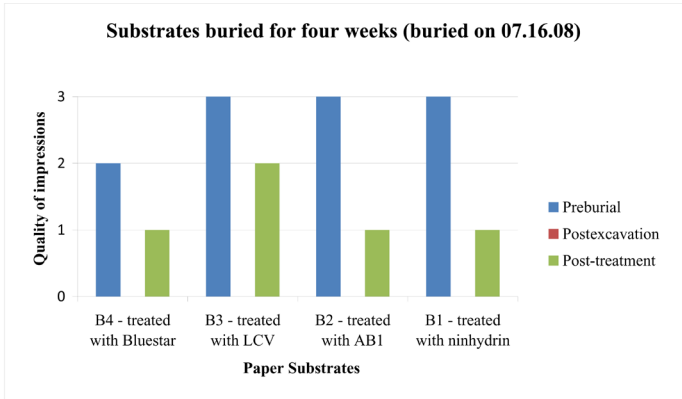


Figure 14

Comparison of paper substrates buried for four weeks.

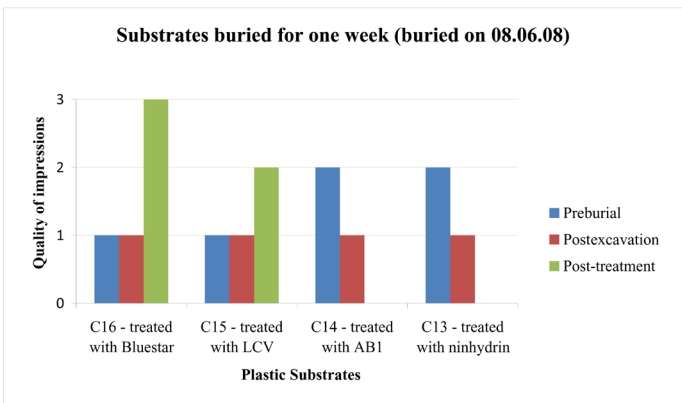


Figure 15

Comparison of plastic substrates buried for one week.

Substrate numbers: C12, C11, C10, C9 (Figure 16)

Recovery of materials on excavation showed no visible signs of impressions after two weeks in the soil, even though they were good preburial. Treatment with ninhydrin and acid black 1 gave no results; LCV and Bluestar gave excellent impressions for comparison purposes.

Substrate numbers: C8, C7, C6, C5 (Figure 17)

Again, poor impressions at the time of burial proved nondistinguishable after three weeks in the soil. The only enhancement method that actually resulted in an impression of good quality was Bluestar.

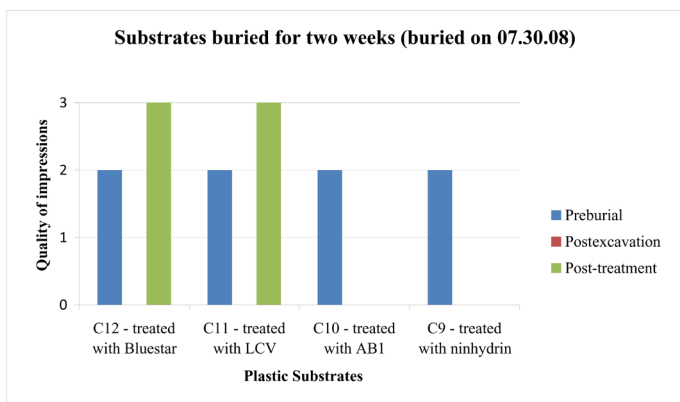


Figure 16

Comparison of plastic substrates buried for two weeks.

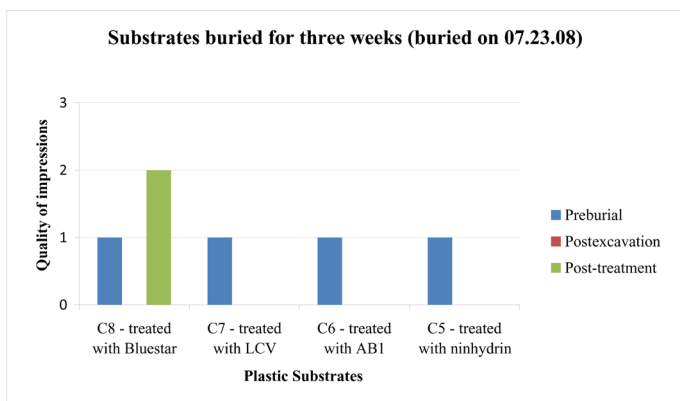


Figure 17

Comparison of plastic substrates buried for three weeks.

Substrate numbers: C4, C3, C2, C1 (Figure 18)

The initial impressions were only of good or poor quality before burial; none were excellent. On recovery of the substrates during excavation, no disintegration had occurred. However, there was no evidence of any visible impressions.

The only enhancement method that worked on plastic recovered after four weeks in the soil was Bluestar, which gave a better quality result than the initial impression.

Bluestar improved from giving good results to impressions after four and three weeks in the soil to giving excellent results after two weeks.

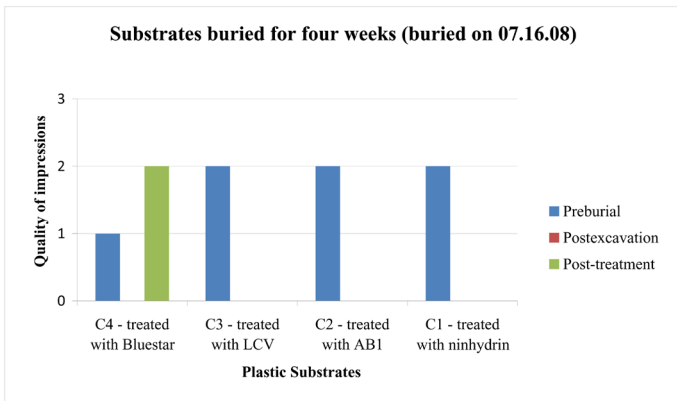


Figure 18

Comparison of plastic substrates buried for four weeks.

Discussion

General observations conclude that more impressions were recovered from the porous substrates than from the nonporous. This suggests that the contaminant (blood) was absorbed into the porous substrate, creating a more visible impression because the blood retained its components within the substrates. Nonporous substrates, however, failed to absorb the blood, and therefore created less-visible impressions. This could be explained by lack of porosity of the substrate, and perhaps, over time, the blood did not absorb into the substrate itself, but rather into the soil with which it was in contact. Further tests to examine this are recommended.

It was clear from the outset that impressions on plastic would prove difficult to recover. Plastic was the only nonporous substrate in the experiment. When creating the impressions, the blood appeared to flake and fall off when dry, because it was not being absorbed into the material. Pooling of blood also occurred on some samples. Color contrast was also an issue because of the dark background color of the material. Therefore, it was clear from the beginning that some enhancement methods would not be suitable.

It can also be noted that on paper substrates, even though some of the impressions and material degraded over time, the typed print on the paper was still legible after four weeks in the soil. This information can be used in investigations to pinpoint locations or dates.

Even though the cotton and paper samples degraded the most over time, it was easier to retrieve impressions from them than from the plastic, which did not deteriorate at all.

Of the 48 initial impressions, 29 were excellent, 12 were good, and 7 were poor. The differences here can be attributed to the application of the impression or by the amount of contaminant on the shoe itself. The most frequently recovered impressions of excellent quality were the first impression in each depletion series. This means that the majority of impressions recovered could be used for comparison purposes against a known impression or searched against a database to identify suspects.

Tables 4 and 5 outline the features of each enhancement method and the advantages and disadvantages associated with each.

	Ninhydrin	Acid black 1	LCV	Bluestar
Nature of Substrate	Very effective on porous surfaces, therefore works well on substrates A and B	Effective on porous and nonporous surfaces, therefore effective on all substrates	Effective on porous and nonporous surfaces, therefore effective on all substrates	Effective on porous and nonporous surfaces, therefore effective on all substrates
Developmental Color	Ruhemann's purple	Blue-black	Deep purple-violet	Chemiluminescence
Ease of Preparation	Special formula spray pump - premixed and ready to use	Aqueous solution, ready to use	Aqueous LCV kit containing aqueous solution of hydrogen peroxide & 5-sulfosalicylic acid, sodium acetate, and LCV. Must be mixed before use	Kit containing 500 mL reagent with safety band, 3 catalyst tablets, and 1 fine mist atomizer. Must be premixed before use.
Method of Application	Spray bottle	Immersion or soak in solution and rinse	Spray bottle	Spray bottle
Costs	Sirchie: 240 ml bottle - \$31.95 (Cat. No: NSI609)	Lightning Powder Co.: 1lt - \$104.95 (Code: 1-2740)	Sirchie: Aqueous LCV Kit - \$32.95 (Cat. No: LV509)	Bluestar Forensic: Kit - £53.80 (Product #BL-FOR-BLUEST)
Source of Product	www.sirchie.com	www.redwop.com	www.sirchie.com	www.bluestar-forensic.com

Table 4

Summary of features associated with each enhancement method.

	Advantages	Disadvantages
Ninhydrin	Can be used before DNA samples are taken* Simple Effective Easy to use Inexpensive	Toxic Requires application of heat Can interfere with forensic examination for DNA profiling
Acid black 1	Can be used before DNA samples are taken* Stable Simple Inexpensive Can be used after the application of LCV**	High background staining Aqueous based formula may sometimes produce diffuse edges, especially on porous surfaces Requires rinsing and may permanently damage substrates Can interfere with forensic examination for DNA profiling
LCV	Fast Safe Uncomplicated Inexpensive Contains fixative No background staining In the event of failure to produce usable evidence, other treatments (e.g., acid black 1) may be used**	May interfere with subsequent blood tests, therefore samples for analyses must be collected prior to application
Bluestar	Does not degrade DNA Fast Extremely sensitive Stable Easy to use Produces bright and long lasting chemiluminescence Can be used as a sequential treatment on all previous treatments, however may not produce successful results**	Gives false positives with bleach, copper, chlorine, metal salts, some paints (but differences in intensity and reaction time allow for visual differentiation by experienced users) Repeated or constant spraying increases amount of fading of chemiluminescence Excessive application can create streaking on vertical surfaces and pooling on horizontal surfaces
<p>* Application before collection of DNA may interfere with forensic examination (see disadvantages). It is recommended to select the single most effective development process appropriate for the surface and immediately submit samples for DNA analysis (22).</p> <p>** The use of a number of sequential treatments is likely to reduce DNA recovery and increase potential for contamination (22).</p>		

Table 5

Advantages and disadvantages associated with each enhancement technique.

Temperature, pH, and Moisture Content

Temperature was recorded throughout the experiment, and an average was taken. Records show that the temperature of the containers was constantly lower than the ambient temperature of 23 °C.

A chemical analysis undertaken ascertained only the soil pH. This was conducted using a hydrogen electrode and three soil samples at the beginning of the experiment. The samples averaged a pH of 6.3, establishing the soil as being one of neutral acidity.

Moisture content of the soil was recorded both at the beginning and the conclusion of the experiment. At the beginning, a sample from each container was tested, and each sample was found to have 15% moisture content. At the conclusion of the experiment, a sample from the box containing substrate A measured 4%, a sample from the box containing substrate B measured 3%, and a sample from the box containing substrate C measured 8%.

Controls

Two sets of controls were assessed following the treatment of all substrates: (1) control samples containing bloody footwear impressions that had not been buried, and (2) control samples of all the substrates that had been buried, with no blood.

Control samples that had been buried with no blood contamination showed the same signs of disintegration and degradation in the early stages of the experiment as those that had been buried with impressions. This suggests that the main reason for degradation was the environment in which the samples were buried, and not the presence of the contaminant.

Cotton and paper control samples that did contain blood contamination but had not been buried showed no sign of loss of impression detail during four weeks. They, therefore, were classed as excellent for the purpose of comparison without the need for enhancement. Again, this shows that the burial environment had an adverse effect on the survival of footwear impressions on substrates, because without it, as can be seen from the controls, no deterioration of material or loss of impression detail occurred. Plastic control samples that were not buried

also showed no signs of deterioration. These were treated with Bluestar, and gave poor to good impressions over the period of four weeks.

All control samples that were buried with no blood were tested with each of the chemicals for signs of false reactions. None of the samples gave any reactions.

Sequencing

It was not the intention of the authors to conduct an experiment examining sequential chemical enhancement treatments of recovered footwear impressions. However, one should take into consideration that employing more techniques in conjunction with one another may result in the recovery of more detailed impression evidence [6]. It is necessary to ensure that tests also do not interfere with subsequent tests to “type” or “group” the blood. “Problems can arise when an entire stain is treated with a reagent that can affect subsequent tests.” [32] Some enhancement methods severely compromise, or prevent altogether, DNA typing. Identifying the correct sequential treatment in both the development and retrieval of developed marks is of paramount importance to prevent the marks being irretrievably damaged or lost [31]. Further tests on sequential processing are recommended.

Results from this study prove, in order of importance, that (1) the length of time in the soil, and (2) material selected are vital factors in the recovery of substrates and impressions from the burial environment.

It is clear that those impressions that were in the soil for a shorter period of time had a better chance of survival. Those that were in the soil for longer became disintegrated and fragmented over time, and this resulted in a loss of impression detail. The authors are of the opinion that there would be great difficulty in retrieving impressions from any of the substrates after a period of four weeks. Also, the longer the substrates were in the soil, the more soil adhered to the material. This became an issue when the substrates were being cleaned prior to treatment. Excess soil resulted in further cleaning. Further cleaning resulted in additional abrasion to the materials, leading to increased damage to the substrate, and therefore a loss of impression detail.

The composition of the substrates also had a vital role to play in the survival of substrates. The substrates that were buried at the early stages of the experiment resulted in a loss of the impression over time. The selected substrates were chosen as common, everyday items, with the potential to be found at crime scenes and crime related burials. Cotton and paper became highly disintegrated after approximately two weeks. Plastic samples remained fully intact throughout the duration of the experiment.

Another factor in the survival of the blood-contaminated impressions was the depth at which they were buried. Impressions that were buried for three and four weeks were buried 5 cm deeper than those buried for one and two weeks. Therefore, further tests are recommended where substrates are buried at the same depth. This will then determine whether it is the depth of the burial or length of time in the soil that is the major contributing factor to disintegration and loss of impression. A difference of 5 cm in this study was deemed to have little effect, and the length of time in the soil was the major factor in assessing the relative survival of impressions.

Moisture content was not controlled for in this study because of difficulties in regulating the amount of water necessary to maintain consistency to observe moisture absorption and moisture evaporation. However, it was measured both at the beginning and conclusion of the experiment. The decrease in moisture content of the soil between the commencement (15% for A, 15% for B, 15% for C) and conclusion (4% for A, 3% for B, 8% for C) of the experiment provides an indication of the evaporation rate. It also suggests an increased rate of moisture absorption with the porous substrates. The highest rate of decrease of moisture content was in containers A and B, the containers containing the porous substrates. Container C (plastic) revealed the lowest rate of decrease of moisture content, suggesting the substrate's lack of porosity and lack of absorption ability and resulting in the lowest rate of visible and recovered impressions throughout the study. Further tests where water can be added to replicate rainfall, or an experiment conducted outdoors, will contribute to further results for this type of study.

An important factor to take into account when assessing the most suitable enhancement technique is the method of application. Those that are applied with the use of a spray bottle are the most effective for use at crime scenes or in the lab.

Ninhydrin, LCV, and Bluestar were applied in the experiment using a spray bottle, and according to results, gave the best impressions overall. Ninhydrin, however, required the application of heat to catalyze a reaction, reducing its practicality for use at crime scenes. LCV and Bluestar required the premixing of certain components. Although this was burdensome, they created the best impressions. However, LCV has a longer shelf life once mixed than Bluestar [27], therefore, the authors recommend LCV as the most efficient enhancement method of blood-contaminated footwear impressions.

Acid black 1 requires the substrate to be immersed in a solution. For some substrates, this may be suitable. However, as can be seen from the results, the water-based solution can be destructive to smaller, weaker substrates and is therefore not recommended by the authors as being of practical use for this type of impression recovery.

Some enhancement methods are more favorable than others, depending on the type of substrate involved, quality of original impression, length of time in the soil, and depth of burial. Many other reagents and formulations exist for the enhancement of blood-contaminated impressions, and further tests are recommended to assess their relative effectiveness on different surfaces.

It is important to take into consideration the background color of the substrate on which the enhancement method is working best. The contaminant chosen was for a specific purpose: it could be easily seen after enhancement. Blood, therefore, will obviously work best on substrates of a lighter background than the contaminant itself. It is therefore recommended to conduct tests with substrates of a similar composition but of a different background color.

Because of the degradation and disintegration of some substrates, UV/ALS examination was not considered necessary. If, however, that material had remained intact, then UV/ALS lighting would have been considered.

Although further DNA testing on the substrates was not addressed in this study, it was important to take into consideration the potential interference with other forensic examinations that could be carried out on the substrates. Any extraneous soil

that was removed from the samples prior to chemical enhancement could be tested for the presence of DNA, as well as the soil with which the substrate had been in contact. It is likely that on removal of the substrate, particles of the contaminant remained on the soil.

The premise that “every contact leaves a trace” has, to some extent, been proven by this experiment. Whether or not impressions were visible on excavation, results after enhancement methods have proven that trace evidence of footwear impressions in blood can survive for up to four weeks in the soil on cotton, paper, and plastic.

However, the ability to produce a positive result for the presence of an impression does not make it of paramount importance to an investigation unless it can be identified. Class, individual, and wear characteristics must be recognized by the examiner and be comparable across either a database of footwear patterns or test patterns. An established number of characteristics on which to positively identify an impression have not yet been determined. However, research currently being undertaken by the Home Office, NPIA, and Forensics 21 is looking toward creating a national footwear database and common standards in the examination of forensic footwear impressions. Standards should be applicable to all those involved in the recovery and presentation of evidence, especially forensic archaeologists and crime scene investigators who are responsible for locating, recovering, excavating, securing, and presenting evidence.

Proposed Guidelines

The authors propose in Table 6 a summary of the effectiveness of chemical enhancement processes across the range of substrates on blood-contaminated footwear impressions based on averages of results achieved over a four-week period.

	Ninhydrin	Acid Black 1	LCV	Bluestar
Cotton	Good	Good	Most effective	Good
Paper	Good	Good	Most effective	Poor
Plastic	No value	No value	Poor	Most effective

Table 6

Summary of effectiveness of chemical enhancement procedures.

From this study, a set of comprehensive proposed guidelines (appendix B) that aim to assist forensic archaeologists and forensic examiners both at the crime scene and in the laboratory in the recovery of footwear impressions made with blood on various substrates within the burial environment has been created.

Conclusion

A controlled experiment was undertaken where 48 identifiable footwear impressions were made with blood on three substrates: cotton, paper, and plastic. These were buried in soil of a neutral type in containers and were excavated at various times during a four-week time period. Of the original 48 substrates buried, 29 (60%) showed visible signs of impressions on excavation.

A systematic evaluation of techniques that react with the components of blood was then carried out. Of the 48 original substrates buried and subsequently excavated, 35 (73%) provided adequate visible impressions identifiable to original impressions following chemical enhancement.

This study concludes that by careful excavation methodology, and with suitable enhancement techniques, there is a potential for a significant amount of blood-contaminated footwear impressions to be recovered from buried substrates. Also, those impressions recovered can be compared with known impressions to prove identification.

Limitations

Because this was a controlled experiment (i.e., the depth and amount of soil was measured, the temperature almost constant, the lighting was controlled, and no rainfall or displacement of substrates by animal activity occurred), the amount of variables that could affect the results were limited.

The use of one soil type and only three substrate types limited the level of results one could hope to achieve using the methods and techniques employed.

Only four enhancement methods were used on only one contaminant. This minimized the possibility of seeing results across a range of development techniques on various contaminants.

Future Recommendations

Although obtaining variable impressions was useful for recognizing depletion effects, an impression quality of an “excellent” grade for all samples at the outset would have increased the number of replications.

This study highlights the need for further research into techniques of recovery, preservation, and potential enhancement of blood-contaminated buried evidence.

It is recommended that the same experiment be conducted in an outdoor environment, in varying soil types and depths, and the effects of temperature and rainfall be assessed.

There is a potential for further experiments using other substrate types, colors, and compositions, as well as numerous development techniques, and a possible sequencing process may be established.

Several studies can be undertaken in a similar manner on a range of contaminants that have the potential to be found at crime scenes (e.g., grease, oil, alcohol, liquids).

Acknowledgment

The authors would like to acknowledge Suzanne Chapman (SSD Forensic Trainer, Hampshire Police) for her input into the initial experimental design. Thanks are also due to Helen Bandey (Higher Scientific Officer, Home Office Scientific Development Branch) and Danyela Kellett (Senior Footwear Analyst, Lancashire Police) for their helpful comments and suggestions regarding this research. The authors would also like to acknowledge and thank the reviewers and editing team of the JFI for their input into producing the final version of this paper.

This research was undertaken as part of the requirement for an MSc in Forensic Archaeology at Bournemouth University, Dorset, United Kingdom.

For further information, please contact:

Siobhan Cullen
1 Ballyraine Park
Letterkenny
Co. Donegal
cullen.siobhan@gmail.com

References

1. Lytle, L. T.; Hedgecock, D. G. Chemiluminescence in the Visualisation of Forensic Bloodstains. *J. For. Sci.* **1978**, *23* (3), 550-562.
2. Doherty, P. E.; Mooney, D. J. Deciphering Bloody Imprints through Chemical Enhancement. *J. For. Sci.* **1990**, *35* (2), 457-465.
3. Bodziak, W. J. *Footwear Impression Evidence – Detection, Recovery, and Examination*, 2nd ed.; CRC Press: Boca Raton, FL, 1999.
4. Bodziak, W. J. Forensic Footwear Evidence. In *Forensic Science: An Introduction to Scientific and Investigative Techniques*, 2nd ed.; Nordby, J. J., James, S. H. Eds.; CRC Press: Boca Raton, FL, 2005; pp 361-376.
5. Bodziak, W. J. Use of Leuco Crystal Violet to Enhance Shoeprints in Blood. *For. Sci. Int.* **1996**, *82* (1), 45-52.
6. Theeuwen, A. B. E.; van Barneveld, S.; Drok, J. W.; Keereweer, I.; Limborgh, J. C. M.; Naber, W. M.; Velders, T., Enhancement of Footwear Impressions in Blood. *For. Sci. Int.* **1998**, *95* (2), 133-151.
7. Ashe, R.; Griffin, R.; Bradford, B. Enhancement of Latent Footwear Marks Present as Grease or Oil Residues on Plastic Bags. *Sci. and Justice* **2000**, *40* (3), 183-187.
8. Sears, V. G.; Prizeman, T. M. Enhancement of Fingerprints in Blood Part 1: The Optimization of Amido Black. *J. For. Ident.* *50* (5) 470-480.
9. Sears, V. G.; Butcher, C. P. G.; Prizeman, T. M. Enhancement of Fingerprints in Blood – Part 2: Protein Dyes. *J. For. Ident.* **2001**, *51* (1), 28-38.
10. Sears, V. G.; Butcher, C. P. G.; Fitzgerald, L. A. Enhancement of Fingerprints in Blood – Part 3: Reactive Techniques, Acid Yellow 7, and Process Sequences. *J. For. Ident.* **2005**, *55* (6), 741-763.
11. SWGTREAD Scope of Work Relating to Forensic Footwear and/or Tire Tread Examiners. *J. For. Ident.* **2005**, *55* (6), 764-765.
12. SWGTREAD Guide for the Preparation of Test Impressions from Footwear and Tires. *J. For. Ident.* **2005**, *55* (6), 781-786.
13. SWGTREAD Guide for the Detection of Footwear and Tire Impressions in the Field. *J. For. Ident.* **2005**, *55* (6), 766-769.
14. SWGTREAD Guide for the Detection of Footwear and Tire Impressions in the Laboratory. *J. For. Ident.* **2005**, *55* (6), 774-777.
15. SWGTREAD Guide for the Collection of Footwear and Tire Impressions in the Field. *J. For. Ident.* **2005**, *55* (6), 770-773.

16. SWGTREAD Guide for the Collection of Footwear and Tire Impressions in the Laboratory. *J. For. Ident.* **2005**, *55* (6), 778-780.
17. SWGTREAD Guide for the Forensic Documentation and Photography of Footwear and Tire Impressions at the Crime Scene. Final 3/2006, www.theiai.org, (accessed September 2008).
18. SWGTREAD Guide for the Examination of Footwear and Tire Impression Evidence. Final 3/2006, www.theiai.org, (accessed September 2008).
19. SWGTREAD Guide for the Chemical Enhancement of Bloody Footwear and Tire Impression Evidence. Final 9/2008, www.theiai.org, (accessed September 2008).
20. Bandey, H. Speculative Application of Protein Stains to Flooring to Enhance Marks in Blood. *Fingerprint and Footwear For. News*. May 2008, Pub. No. 24/08, 11-15.
21. Trozzi, T. A.; Schwartz, R. L.; Hollars, M. L. *Processing Guide for Developing Latent Prints*; Federal Bureau of Investigation, Laboratory Division, U.S. Department of Justice, U.S. Government Printing Office: Washington D.C., 2001.
22. Kent, T., Ed. *Manual of Fingerprint Development Techniques*, 2nd ed. 2nd rev; Home Office, Police Scientific Development Branch: Sandridge, UK, 2004.
23. Latent Fingerprint Processing Techniques - Selection and Sequencing Guide. Chesapeake Bay Division Website. www.cbdi.ai.org/Reagents/indane.html, (accessed 15 September 2008).
24. Aqueous Leucocrystal Violet Technical Information (1-2736). Lightning Powder Company Inc., 2008. Available from www.redwop.com, (accessed 15 September 2008).
25. Spence, L.; Asmussen, G. Spectral Enhancement of Leucocrystal Violet Treated Footwear Impression Evidence in Blood. *For. Sci. Int.* **2006**, *132* (2), 117-124.
26. Miller, R. A. Leuco Crystal Violet: A Simple, Effective, Blood Enhancement Reagent. *The Print*, **1996**, *12* (4), 11. Available from: www.scafo.org/library/120405.html, (accessed 15 September 2008).
27. Aqueous Leuco Crystal Violet Technical Information (LV509). SIRCHIE 2008 Product Catalog. Available from www.sirchie.com, (accessed 15 September 2008).
28. *Bluestar Forensic Latent Bloodstain Reagent User's Manual*. Bluestar: Monte Carlo, Monaco, 2006. Available from: www.bluestar-forensic.com, (accessed 15 September 2008).

29. Young, T. A Photographic Comparison of Luminol, Fluorescein, and Bluestar. *J. For. Ident.* **2006**, 56 (6), 906-912.
30. Dilbeck, L., Use of Bluestar Forensic in Lieu of Luminol at Crime Scenes. *J. For. Ident.* **2006**, 56 (5), 706-720.
31. Chapman, S. *A Study into the Survivability, Development and Identification of Fingerprints from a Burial Environment Utilising Vacuum Metal Deposition and the National Automated Fingerprint Identification System, IDENT1*. Unpublished M.Sc. Dissertation, School of Conservation Sciences, Bournemouth University, 2006.
32. Winchester, R.V. Blood Detection by Chemical Methods. XII-Biotech-A-Blood Detection. Yr. Unknown. Available from: www.nzic.org.nz/ChemProcesses/biotech, (accessed 18 September 2008).

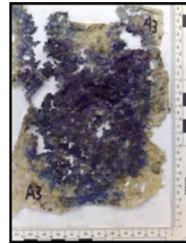
Appendix A



Preburial



Postexcavation



Post-treatment

A weathered and disintegrated cotton substrate (A3), having spent 4 weeks in the soil, is treated with LCV and shows some signs of retrieving the impression.



Preburial



Postexcavation



Post-treatment

A recovered paper substrate (B11) from the soil after 2 weeks is treated with LCV and shows excellent signs of gaining information from the impression.



Preburial



Postexcavation



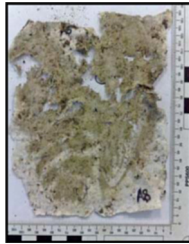
Post-treatment

Plastic substrate (C11) showing no visible signs of the impression upon recovery from the soil after 2 weeks is treated with LCV and the impression is now visible.

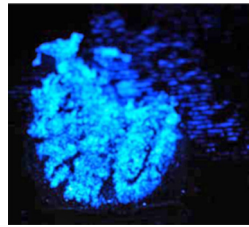
Appendix A (continued)



Preburial



Postexcavation



Post-treatment

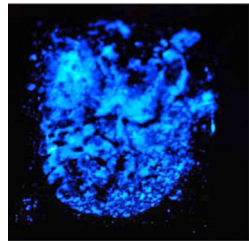
Bluestar treatment on a cotton substrate (A8) that was in the soil for 3 weeks proved useful.



Preburial



Postexcavation



Post-treatment

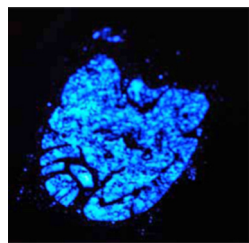
Plastic impression (C4) that showed no visible sign of the impression on recovery after 4 weeks in the soil, was treated with Bluestar and showed very good signs of the impression.



Preburial



Postexcavation



Post-treatment

Bluestar was useful on most of the plastic substrates. Here it retrieved another impression on C12 after 3 weeks in the soil.

Appendix B

Guidelines for the excavation, recording, and enhancement of footwear impressions made with human blood on white cotton, newspaper, and black plastic trash bags from the burial environment.

1. A photographic record must be made at all times during the excavation, recording, and enhancement of the footwear images.
2. Careful excavation techniques must be employed when locating evidence from a burial environment.
3. Once the substrate has been located, it is essential to ensure damage does not occur when lifting. This may be difficult in some situations (e.g., when the substrate has been in the soil for a period of time, disintegration may have occurred) and therefore careful location and lifting techniques must be applied.
4. Once the substrates have been excavated, they must be left to dry before any further treatment.
5. Any extraneous soil can be removed by light brushing.
6. Soil which has been removed should be retained for further analysis.
7. Two major factors affecting the choice of enhancement method include nature of the substrate and background color of the substrate.
8. It is important to consider taking DNA samples before the application of enhancement methods, because some may interfere with subsequent blood tests (Table 4 and 5).
9. The application of leucocrystal violet is safe, stable, and cost-effective and proves most efficient for blood-contaminated porous substrates of white cotton and paper.
10. The application of Bluestar is simple and fast and is most effective for enhancing blood-contaminated impressions on nonporous black plastic trash bags.