

Chemical Enhancement of Footwear Impressions in Blood Recovered from Cotton using Alginate Casts

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Abstract: Depletion series of footwear impressions in blood were deposited on black cotton fabric after which they were lifted using alginate and subsequently enhanced using protein stains amido black (AB), Crowle's stain (CS), coomassie blue (CB), and Hungarian red (HR). Other factors that were considered during this study were the age of the impression and the temperature of the environment. A novel score system for the enhancement of footwear impressions was introduced, which used the product of scores for size and detail of the impression.

The study showed that temperatures between 8 °C and 37 °C did not impact chemical enhancement, whereas the age of the impression did. An impression aged for 7 days yielded higher enhancement scores than impressions aged for 1 or 28 days, especially for AB. The results of depletions 1 to 5 were similar to the results of only depletion 5. However, at depletion 5, AB was the best-performing protein stain.

CB and AB yielded the highest level of enhancement of the impressions, whereas CS and HR resulted in poorer quality enhancements. AB was the preferred protein stain of use because AB was the most sensitive protein stain used in this study and there were fewer health risks involved in using water-based AB than in using methanol-based CB.

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Introduction

At crime scenes, the identification of footwear impression evidence may be challenged by the color of the substrate on which the impression was deposited. The impression cannot always be recovered for analysis in the laboratory and, as a result, photography is often the only means of recovering the evidence [1]. Ultraviolet (UV) detection and subsequent photography are commonly used for the detection of bloodstains, because blood has a strong light absorption from 300 to 900 nm and a narrow absorption peak near 415 nm. Suggestions have been made for the use of infrared (IR) light for the detection of bloodstains, although both UV and IR techniques require a contrast in the absorption of the background substrate and the bloodstain to enable visualization [2, 3]. Normal lighting conditions are also used for the photography of evidence. In some cases, photography under UV, IR, or normal lighting conditions does not yield the desired results because of an interfering color of the substrate on which that the impression was made [4].

Lifting techniques, such as the electrostatic lifting apparatus (ESLA), adhesive lifters, gelatin lifters, or alginate casts, could be used to transfer the impression onto a substrate that may offer a better contrast for photography [4–6]. Depending on the lifting method, the transferred impression may then be chemically treated to increase detail and impression size, if required.

Alginates are water-soluble polysaccharide biopolymers extracted from brown seaweed, and they are mainly used for their gel-forming properties [7]. They are commonly used by dentists for making dental casts because of their safety, quick preparation time, quick setting time, and their flavor. It has been suggested that alginates could be used for the recovery of footwear impressions, although the use of this material for the recovery of footwear impressions has not been studied in depth [8, 9]. Alginates have been used for the recovery of bloody footwear impressions from concrete, fabric, and human skin and have been subsequently enhanced using leucocrystal violet (LCV) [8] and amido black [9]. A recent study shows that alginates can serve as a lifting technique for fingerprint impressions in blood, although the fine ridge detail is difficult to lift from substrates such as denim, glass, and paper, and, therefore, the use of alginates for fingerprint lifting should be studied more in depth [10]. The main drawback of the use of alginates is that alginates tend to shrink as they dry, and for this

reason, alginates need to be photographed shortly after chemical enhancement to prevent loss of detail [8–12].

The current study is based on previous research by Farrugia [12] who determined the effect of various enhancement chemicals on footwear impressions on several fabrics. The footwear impressions were made using contaminants such as blood, urine, and soil [13–17].

In this study, depletion series of footwear impressions in blood were made on black cotton using a semi-automated stamping device developed by Farrugia [12, 13, 18]. The depletions were stored at different temperatures for different periods of time to determine the effect of temperature and age on the impressions.

Four protein stains were chosen to chemically enhance the bloody footwear impressions: amido black (AB, acid black 1), Crowle's stain (CS, crocein scarlet 7B mixed with coomassie blue), coomassie blue (CB), and Hungarian red (HR, acid fuchsin). These protein stains were chosen because of the CAST recommendation for the use of AB on blood [19], the suggestion for the use of these protein stains for visualization of marks in blood on the BVDA website, and the capability of AB and the other stains to enhance the impression without the need of special lighting equipment [20]. Several studies investigated the use of AB [12, 16, 21–24], CS [21, 24], CB [21, 24], and HR [21, 24] for the visualization of footwear and fingermark impressions in blood. Hungarian red also has fluorescent properties on application of a 100 times diluted HR reagent to a gelatin lifter [24, 25]. However, the current study only investigates visual enhancement.

The sensitivity and efficacy of the protein stains were compared by grading the size of the impressions and the amount of detail visible, both before and after enhancement.

Materials and Methods

Preparation of the Depletions

The depletions were prepared using a semi-automated stamping device (Figure 1). This stamping device was designed and built during a previous study [18] to simulate the average stamping force of men and women (3500 N) and to minimize the variability of the stamping force. It consists of a wooden foot that holds the shoe, which is attached to a weight of 10.2 kg to

achieve a stamping force of approximately 3500 N. The device is designed to lift the shoe up to a height of 0.64 m after which it is dropped, using a release mechanism on the device.

The shoe that was used during this study was a trainer manufactured by Etnies. This particular shoe was chosen for the variety of patterns on the sole (Figure 2). A piece of black cotton fabric (100% cotton, size 0.20 m x 0.40 m) was placed underneath the stamping device on top of high-density foam (0.10 m thick). This fabric was chosen for the deposition of the bloody footwear impression because the contrast between the blood and the fabric would be poor. Therefore, a lifting method should be used to obtain most of the detail and contrast from the impression. A metal tray (0.36 m x 0.25 m x 0.04 m) was filled with a Kimberly double ply paper towel and 21 to 31 mL of human blood. The sole of the shoe was placed inside the metal tray, coming into contact with the paper towel and ensuring that all parts of the sole had been covered with blood. A footwear impression in blood was prepared by utilizing the release mechanism on the stamping device, after which the impression was outlined using a white pencil. The cotton was then removed from the device, after which a new clean cotton sheet was placed underneath the device. The device was again set to its maximum height, and another footwear impression was prepared. This process was repeated four times to create a depletion series of five impressions in total because of the findings of Farrugia et al. [12, 13, 16], which showed that there was little enhancement after the fifth depletion. After a series had been prepared, the shoe was removed from the device and cleaned thoroughly using Trigene disinfectant and a brush.



Figure 1

*The semi-automated stamping device used in this study [13]
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Figure 2

The sole of the Etnies shoe used in this study.

The Impression Variables

A total of 45 depletions (9 depletion series) were prepared during this study. Three depletion series were stored on a table in the laboratory at room temperature (approximately 20 °C), three depletion series were stored in the refrigerator (approximately 8 °C), and three depletion series were stored in the oven (approximately 37 °C). These different temperatures were chosen to mimic natural environmental conditions in which a footwear impression could be deposited and aged. However, besides temperature, other environmental conditions exist (e.g., wind and humidity), which were not controlled in this study.

From all series stored at different temperatures, one series was aged for 1 day, one series was aged for 7 days, and one series was aged for 28 days. For each variable, negative and positive controls were prepared. The negative controls were prepared by leaving a footwear impression on black cotton using the shoe that was not covered in blood, ensuring the sole was dry. Another series of negative controls was prepared using the shoe sole covered in distilled water. Positive controls were prepared following the standard protocol for the preparation of the first depletion. Each depletion series contained one dry negative control, one wet negative control, and one positive control impression for each condition to which the impressions were subjected. Figure 3 shows a diagram of the variables to which the depletion series were subjected. The diagram excludes chemical treatment.

Recovery of the Bloody Footwear Impressions

The bloody footwear impressions were recovered using GC Aroma Fine Plus alginate (GC Europe, Leuven, Belgium). The alginate was prepared by mixing six scoops of alginate powder (approximately 49.8 g) with 134 mL of tap water. The mixture was then stirred for 30 seconds after which it was applied onto the bloody footwear impression on the black cotton, using a spatula and ensuring that a consistent layer was produced. It was left to dry for 5 minutes before it was lifted from the cotton, cut into quarters using a scalpel, and labeled by scratching a code into the back of the alginate, using a needle.

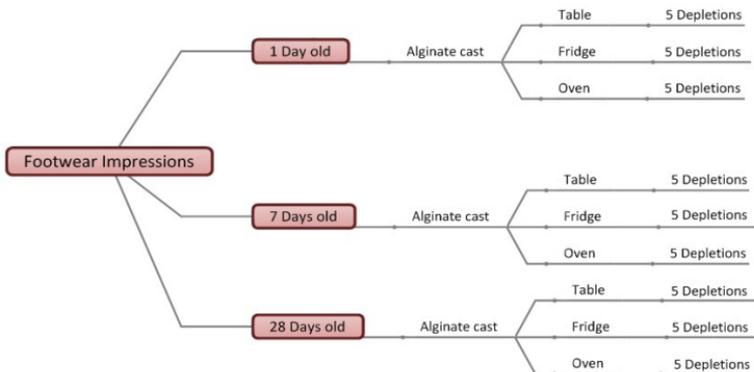


Figure 3

Diagram of the depletion series and the variables to which they were subjected.

Chemical Enhancement

Prior to enhancement of the quartered impressions with the protein stains, a fixation step was included for two reasons: (1) some of the protein stains were water-based, which may have caused the blood to dissolve in the protein stain, (2) the blood could have dispersed through the water-based alginate, which would leave a blurry impression. The most common fixation method is immersion of the impressions in a 2% 5-sulphosalicylic acid in water solution, which precipitates the basic proteins and thereby prevents diffusion [19].

Different immersion times are used in the literature, ranging from 2 minutes [24] to 5 minutes [12–16], and in two studies where AB was used, no fixation step was applied [9, 21]. Therefore, a small preliminary test was conducted to determine the effect of fixation time on the impression and it was concluded that a 1-minute fixation time was sufficient for the bloody impression not to disperse throughout the alginate. After subsequent chemical enhancement, there was no sign of blurry impressions resulting from insufficient fixation.

Each alginate quarter was fixed for 1 minute using a 2% 5-sulphosalicylic acid in water solution (BVDA, Haarlem, The Netherlands) and was subsequently immersed for 5 minutes in baths of either AB (water based), CB (methanol based, conducted in a fumehood), CS (water based, containing trifluoroacetic acid), or HR (water based) (all protein stains were

premixed from BVDA, Haarlem, The Netherlands) [12]. They were then left to dry for 5 minutes and destained with tap water for 1 minute. The alginate quarters were photographed in natural lighting approximately 15 minutes after destaining to minimize loss of detail because of the shrinkage of the alginate. A Canon EOS 600d camera was used, which was positioned at a 90° angle to the impressions.

Amido black is commercially available in different formulations [e.g., methanol, water, or water–ethanol–acetic acid (WEAA)]. The formulation that is currently recommended by CAST is the WEAA formulation, however this formulation is known to cause background staining [22] and, because of availability and safety reasons, the water-based AB was used for this study.

Grading the Impressions

The photographs of the alginate quarters were graded using the grading tables presented in Tables 1 and 2. Each quarter of an impression was assigned a score from 1 to 5, before and after enhancement, to determine the degree of enhancement of the visible proportions (size) and the amount of detail visible in the impression, yielding a total of four scores for each quarter impression: two scores (size and detail) before enhancement and two scores (size and detail) after enhancement. Two scores were calculated (one before, one after) by multiplying the size (before) with the detail (before), and the size (after) with the detail (after), ranging from 1 (1 x 1) to 25 (5 x 5). This larger scale allows a better overall characterization of the footwear impressions. Finally, the combined “before” score was subtracted from the combined “after” score, resulting in one final score for the level of enhancement of each quarter impression, ranging from 0 to 24.

The grading scales used in previous studies [12, 24] were used to assign a score to the overall enhancement of the impression. These scales did not differentiate between the enhancement of the impression in size and the enhancement of footwear detail, and, therefore, it was difficult to interpret those results. The grading tables used in this study were based on a fingerprint grading system [26], however, using a score of 0 was inappropriate because information of a partially visible impression would be lost in the product of the score for the size and the score for the amount of detail visible. For this reason, the grading scale was adjusted from 0 to 4, to 1 to 5.

The use of the proposed combined score for the size and detail of the impression has three advantages: (1) it combines the two important characteristics of the impressions (i.e., the visible proportions of the size and the amount of detail visible), (2) it allows a good overall characterization of the enhancement of the impressions because of its larger grading range, (3) it allows the calculation of one final enhancement score for each quarter impression for each factor combination.

Grade	Proportion of the Impression Visible
1	No evidence of impression
2	0–1/3 of the impression visible
3	1/3–2/3 of the impression visible
4	2/3–1 of the impression visible
5	Full impression is visible

Table 1

Grading scale with regard to the size of the impression in the alginate quarter.

Grade	Proportion of Detail Visible
1	No detail visible
2	0–1/3 of the detail visible
3	1/3–2/3 of the detail visible
4	2/3–1 of the detail visible
5	All detail is visible

Table 2

Grading scale with regard to the detail of the impression visible in the alginate quarter.

Statistical Analysis

Using IBM SPSS Statistics 22, the analysis of variance (ANOVA) was calculated for the enhancement score as response variable for depletions 1 through 5 to determine whether there were any significant effects between the three factors (age, temperature, and protein stain). In this three-way ANOVA test, $p < 0.05$ indicated that the variable had a significant effect on the response, $0.05 \leq p < 0.10$ indicated a borderline significant effect, and $p \geq 0.10$ indicated that the variable did not have a significant effect on the response. The response in this study would be the final enhancement score calculated for each quarter of all impressions.

Then, main effects plots and interaction plots were prepared to visualize these effects. Main effects plots display the enhancement score for each variable, whereas interaction plots display the enhancement score of the interactions between two variables. These plots were used to interpret the differences between levels of (borderline) significant effects.

To determine which of the effects are significant at the end of a depletion series, ANOVA results and graphical output are also given for depletion 5. By doing so, the sensitivity of the four reagents is compared.

Results and Discussion

Chemical Enhancement of Alginates

The negative controls showed no evidence of blood after alginate lifting followed by chemical enhancement. All positive controls were chemically enhanced and ensured proper working of all four protein stains. Figure 4 shows depletions 1, 3, and 5 before enhancement which were aged for 7 days and stored on a table in the laboratory at 20 °C. Figure 5 shows the depletions after subsequent enhancement. As can be seen in the figures, the fifth depletion impression before enhancement shows very little of the characteristics of a footwear impression and the detail is not visible. The chemical enhancement of this impression shows much more detail, especially the upper left quarter enhanced by amido black, of which a large amount of detail of the footwear impression can be easily identified. In contrast, the fourth reagent at the bottom right (Hungarian red) does not enhance the fifth depletion footwear impression.

The first depletion was overloaded with blood, and, therefore, the blood seems to have dispersed throughout the alginate. Because blood dissolves in water and alginates are water based, it is reasonable to assume that when more blood is present, more blood will dissolve into the alginate. This causes blurring of the impression that occurs before the proteins are fixed with 2% 5-sulphosalicylic acid, and the visible detail could not be improved by chemical enhancement.

Despite controlling the variable of pressure using the semi-automated stamping device, there was a difference between the amount of blood deposited onto the fabric (Figure 6), which presents alginate lifts of three first depletions stored for 28 days at 8 °C, 20 °C, and 37 °C, respectively, before enhancement.

There are clear differences between the three impressions in regards to the amount of blood present, hence, three first depletions can be assigned different scores for the size and detail before enhancement. Figure 7 presents the enhanced alginate lifts, which shows that there is a difference in the level of enhancement.

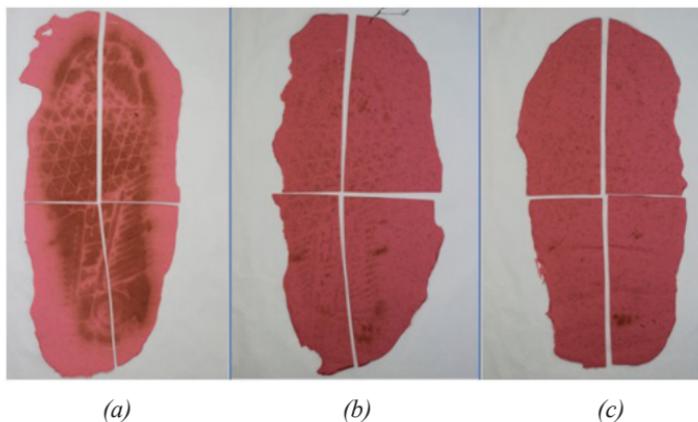


Figure 4

Alginate lifts of depletions 1 (a), 3 (b), and 5 (c) before enhancement, which were stored for 7 days at 20 °C.

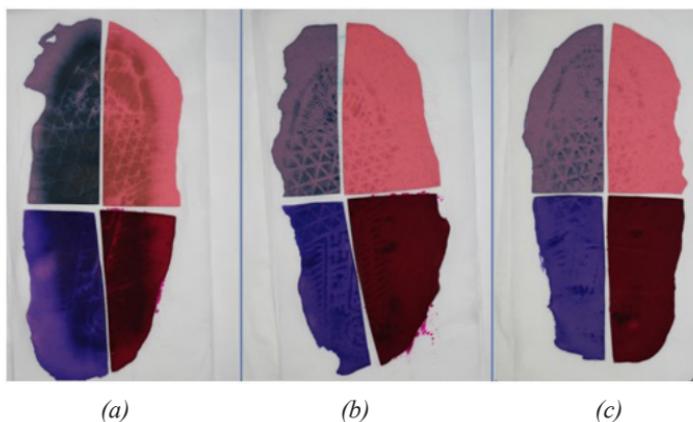
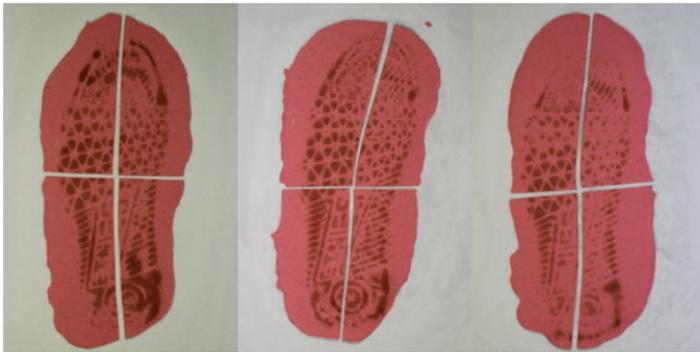


Figure 5

Alginate lifts of depletions 1 (a), 3 (b), and 5 (c) after enhancement, which were stored for 7 days at 20 °C. The upper left quarters were enhanced with AB; the upper right quarters were enhanced with CS; the lower left quarters were enhanced with CB; and the lower right quarters were enhanced with HR.



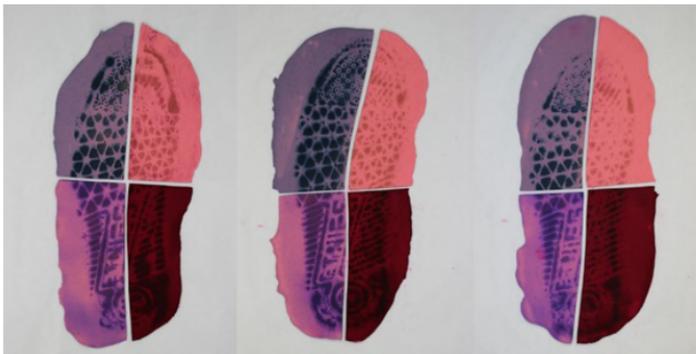
(a)

(b)

(c)

Figure 6

Alginite lifts before enhancement of depletion 1, which were stored for 28 days at 8 °C (a), 20 °C (b), and 37 °C (c).



(a)

(b)

(c)

Figure 7

Alginite lifts after enhancement of depletion 1, which were stored for 28 days at 8 °C (a), 20 °C (b), and 37 °C (c).

Contrast of the Enhanced Impressions

One important aspect of the enhancement of the impression is contrast, however, this variable is not accounted for in the grading tables. The results show that background staining is a more prevalent problem using HR. Figure 8 shows depletion 4 stored at 8 °C for 1 day, which is enhanced using AB, and depletion 4 stored at 8 °C for 1 day, which is enhanced using HR. Both of the impressions were graded 1 in size and detail before enhancement (product 1 x 1 = 1) and graded 2 in size and detail after enhancement (product 2 x 2 = 4), resulting in a final enhancement score of 3 (4 – 1 = 3).

The figure shows that contrast is a crucial variable. To identify the enhancement that HR has yielded, one has to look very closely at the impression to determine that a part of the impression is vaguely visible in the upper and lower left corner. Visualizing the actual mark is complicated by background staining of the alginate, which is less of a problem for the enhancement using AB, as seen on the right side of Figure 8. The background staining is not a major problem in this case and the size and some vague detail can be easily identified.

Bialek [27] demonstrated that HR yields the best enhancement of impressions in blood when used on nonporous substrates. If it is used on porous substrates, however, background staining occurs, which complicates visualization of the impressions. The results Bialek found are consistent with the results found in this study.

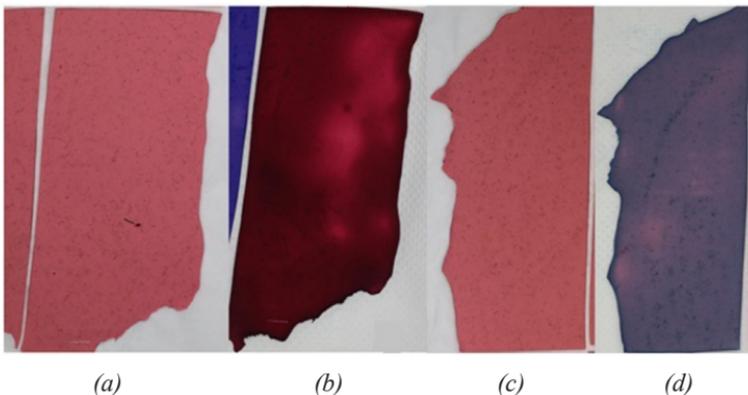


Figure 8

Quarter impression of depletion 3, aged for 1 day at 8 °C before (a) and after (b) enhancement with HR; quarter impression of depletion 4, aged for 1 day at 8 °C before (c) and after (d) enhancement with AB.

Statistical Analysis

ANOVA Results of Depletions 1 to 5

Three-way ANOVA tests were performed on the enhancement scores to determine whether there were significant main or interaction effects of the age, temperature, and protein stain variables used in this study. Table 3 shows the ANOVA results of the enhancement scores of depletions 1 to 5 combined.

Table 3 shows that there is a significant main effect of the age of the impression and the protein stain to which the impressions are subjected. The temperature is borderline significant ($p = 0.071$) and the interaction of age multiplied by temperature ($p = 0.059$) is borderline significant. These effects are investigated further by main and interaction effect plots.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1323.394 ^a	35	37.811	1.863	.006
Intercept	3183.606	1	3183.606	156.892	.000
Age	303.144	2	151.572	7.470	.001
Stain	491.128	3	163.709	8.068	.000
Temperature	109.078	2	54.539	2.688	.071
Age * Stain	96.989	6	16.165	.797	.574
Age * Temperature	188.922	4	47.231	2.328	.059
Stain * Temperature	17.322	6	2.887	.142	.990
Age * Stain * Temperature	116.811	12	9.734	.480	.924
Error	2922.000	144	20.292		
Total	7429.000	180			
Corrected Total	4245.394	179			

^aR squared = .312 (Adjusted R squared = .144)

Table 3

Tests of between-subjects effects. ANOVA results for depletions 1 to 5.

Main Effect Plots of Depletions 1 to 5

Figures 9, 10, and 11 present the main effect plots for the enhancement score of the quarter impressions at a different age, temperature, or by using a different protein stain. Figure 9 includes the average enhancement score for quarter impressions stored for 1 day (1), 7 days (2), and 28 days (3). One assumption that was made was that as the aging period increased, the impressions would deteriorate, and, therefore, enhancement of older impressions would yield poorer quality results than enhancement of fresh impressions, where proteins have degraded minimally. All impressions aged for 7 days were significantly more enhanced than the impressions aged for 1 day and 28 days. This may be due to the fact that the 1-day aged impressions may not have dried sufficiently for the alginate to lift them, and that after 28 days, proteins had started to degrade, which reduced the level of enhancement.

Figure 10 includes the average enhancement score for quarter impressions stored at 8 °C (1), 20 °C (2), and 37 °C (3). One of the assumptions that was made was that the refrigerator would have a preserving effect on the bloody impressions, whereas the warm temperature of the oven would cause faster degradation of the proteins, and, therefore, the enhancement of these impressions would be complicated because of deterioration of the original impressions. Figure 10 shows that this trend indeed exists and that there is a difference between the level of enhancement of impressions stored in environments with different temperatures. However, the ANOVA results indicate that this difference is not significant.

Figure 11 includes the average enhancement score for quarter impressions enhanced using AB (1), CS (2), CB (3), and HR (4). There is a clear difference between the four protein stains: AB and CB yielded excellent enhancement scores, and CS and HR performed poorly.

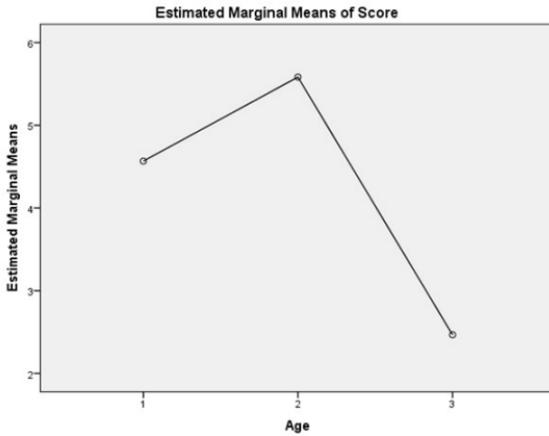


Figure 9

Main effect plot of the age of the impressions. The X axis represents 1-day-old (1), 7-day-old (2), and 28-day-old (3) impressions; the Y axis represents the enhancement score of the impressions from depletions 1 to 5.

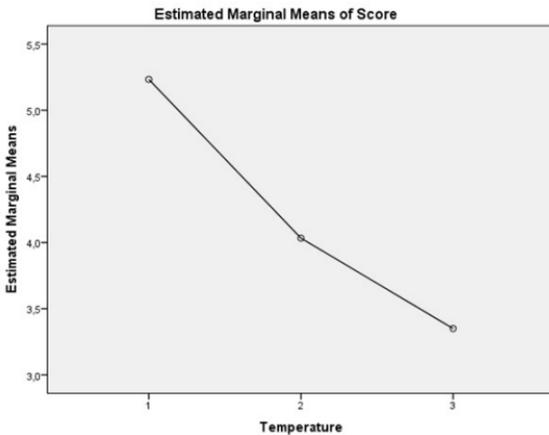


Figure 10

Main effect plot of the temperature at which the impressions were stored. The X-axis represents 8 °C (1), 20 °C (2), and 37 °C (3); the Y-axis represents the enhancement score of the impressions from depletions 1-5.

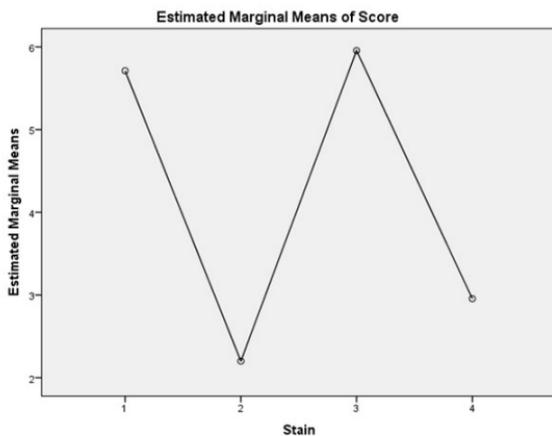


Figure 11

Main effect plot of the protein stains that were used to enhance the impressions. The X-axis represents AB (1), CS (2), CB (3), and HR (4); the Y axis represents the enhancement score of the impressions from depletions 1 to 5.

Interaction Plots of Depletions 1 to 5

Interaction plots were prepared to show the possible interactions between variables that may affect the enhancement of impressions. Figure 12 displays the interaction plot of the age of the impression and the protein stain used, where the averaged enhancement scores of depletions 1 to 5 at all temperatures are the response. The figure shows that in general, protein stain CB (3) resulted in the highest enhancement scores except for the 1-day aged impressions, which yielded a higher level of enhancement when AB (1) was used. Both CS (2) and HR (4) yielded low enhancement scores. These results correspond to the best levels 1 and 3 of the main effect of protein stains (Figure 11). The expectation would be that as more time passed, less enhancement would be achieved because of degradation of the proteins in the blood. CS shows the pattern that would be expected for this interaction. The other protein stains show a rise in the level of enhancement when the impressions have been aged for 7 days. The differences shown in this interaction plot are not significant.

Figure 13 displays the interaction plot of the age of the impression and the temperature at which the impression was stored, where the averaged enhancement scores of depletions 1 to 5 for all protein stains are the response. This effect is borderline significant and in general, impressions yielded the highest enhancement scores for this interaction effect when stored at 8 °C, which corresponds to the best level of the main effect of temperature (Figure 10).

Surprisingly, the impressions that were aged for 1 day and stored at 37 °C yielded a higher level of enhancement than the impressions stored for 1 day at 8 °C and 20 °C. This may have been due to the drying process of the blood (i.e., the blood dries faster at 37 °C than it does at 20 °C or 8 °C). This could indicate that alginate lifting works better on a dried impression than a wet impression, which would imply that 1 day is not sufficient for the bloody impression to fully dry at 20 °C and 8 °C. Another variable that was not controlled during this study was the humidity, which was higher in the refrigerator and on the table than in the oven, where the environment was much drier.

The interactions between protein stains and aging period (Figure 12), and aging period and temperature (Figure 13), clearly showed that in general, the enhancement level of the 28-day impressions was less than the enhancement level of the 1-day impressions. The expectation was to see the enhancement level of the 7-day marks to be within these upper and lower

limits, although this was not the case. These results are consistent with the finding that 7 days is the best level of the main effect of age (Figure 9).

Figure 14 displays the interaction plot of the temperature at which the impression was stored and the protein stain with which the impression was treated, where the averaged enhancement scores of depletions 1 to 5 for all ages are the response. These differences are not significant. The enhancement scores for AB (1) and CB (3) were high, however, whenever impressions were stored at 8 °C, CB yielded the highest enhancement scores of all protein stains.

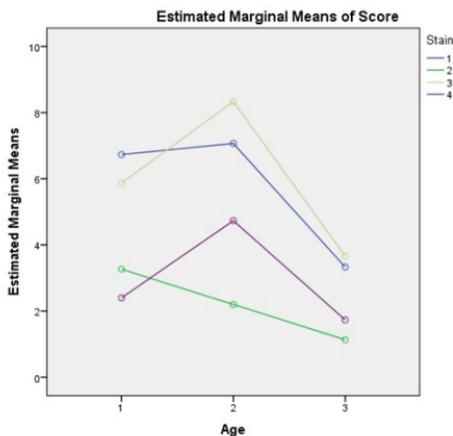


Figure 12

Interaction plot of the variables age times protein stain. The X axis represents 1-day-old (1), 7-day-old (2), and 28-day-old (3) impressions; the Y axis represents the enhancement score of the impressions from depletions 1 to 5. The results for the level of enhancement for each protein stain are displayed: stain 1 is AB (blue), stain 2 is CS (green), stain 3 is CB (yellow), and stain 4 is HR (purple).

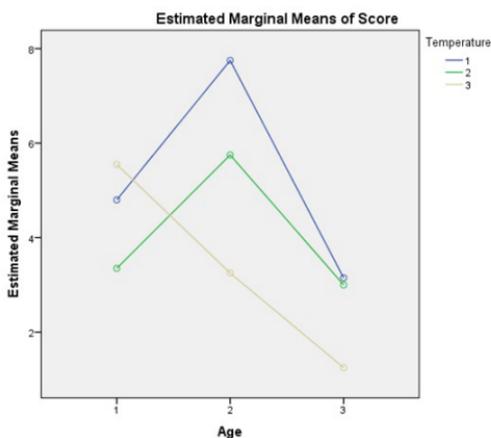


Figure 13

Interaction plot of the variables age times temperature. The X axis represents 1-day-old (1), 7-day-old (2), and 28-day-old (3) impressions; the Y axis represents the enhancement score of the impressions from depletions 1 to 5. The result is the level of enhancement for each temperature variable: 1 is 8 °C (blue), 2 is 20 °C (green), and 3 is 37 °C (yellow).

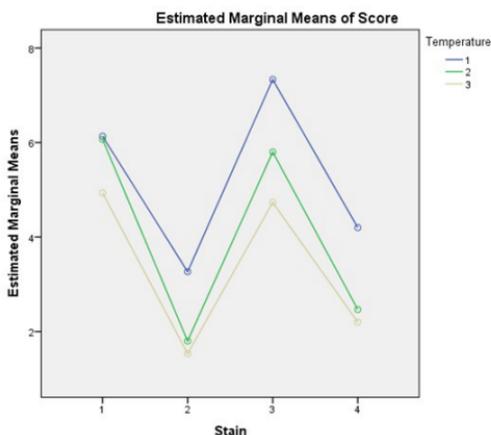


Figure 14

Interaction plot of the variables protein stain times temperature. The X axis represents the protein stains: stain 1 is AB (blue), stain 2 is CS (green), stain 3 is CB (yellow), and stain 4 is HR (purple). The Y axis represents the enhancement score of the impressions from depletions 1 to 5; the result is the level of enhancement for each temperature variable: 1 is 8 °C (blue), 2 is 20 °C (green), and 3 is 37 °C (yellow).

Statistical Analysis of Depletion 5

To determine the influence of the variables on the scores of final depletion 5, a 3-way ANOVA test was also conducted for all depletion 5 enhancement scores. The results (Table 4) indicate that there are significant main effects of the variables age and protein stains. Additionally, significant interaction effects between both age and protein stains, and age and temperature, exist. The results of depletion 5 correspond to the results of all depletions combined. However, minor differences exist, which are presented hereafter.

Figure 15 shows the main effect plot of the protein stains and their corresponding scores for depletion 5. Despite the low enhancement scores compared to the scores of all depletions 1 to 5, AB yielded the highest level of enhancement. Table 5 shows a comparison of the ANOVA results of depletions 1 to 5 with the ANOVA results of depletion 5. In both datasets of depletions 1 to 5 and only of depletion 5, there is a significant effect of the age of the impression: impressions aged for 7 days yielded higher enhancement scores and their main effects plots show similar trends.

The ANOVA of depletions 1 to 5 shows that AB and CB yielded the best level of enhancement. However, the plot of main effect protein stain of depletion 5 (Figure 15) shows that AB is the best protein stain. Because AB only yielded an average enhancement score of 2.5, the investigation into further depletions 6 or 7 will not yield many useful results.

The differences in enhancement score of depletions 5 stored at different temperatures were not significant, whereas the differences in enhancement score of depletions 1 to 5 at different temperatures were larger and borderline significant. This is explained by the small amount of blood present in the impression of depletion 5, which results in a low enhancement score range, which is larger for the averaged depletions 1 to 5, and, therefore, larger differences exist between scores.

For depletion 1 to 5, the interaction effect of age and temperature is borderline significant, whereas this is not the case for only depletion 5.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	151.278 ^a	23	6.577	4.751	.004
Intercept	40.111	1	40.111	28.977	.000
Age	53.722	2	26.861	19.405	.000
Stain	30.778	3	10.259	7.411	.005
Temperature	2.389	2	1.194	.863	.447
Age * Stain	34.722	6	5.787	4.181	.017
Age * Temperature	28.278	4	7.069	5.107	.012
Stain * Temperature	1.389	6	.231	.167	.981
Error	16.611	12	1.384		
Total	208.000	36			
Corrected Total	167.889	35			

^aR squared = .901 (Adjusted R squared = .711)

Table 4

Tests of between-subjects effects. ANOVA results for depletion 5.

Depletion 1-5			Depletion 5		
Significant ($p < 0.05$)	p	Optimal Setting	Significant ($p < 0.05$)	p	Optimal Setting
Age	0.001	2	Age	0.000	2
Stain	0.000	1 + 3	Stain	0.005	1
			Age * Stain	0.017	
			Age * Temperature	0.012	
Borderline Significant ($0.05 < p < 0.10$)	p	Optimal Setting	Borderline Significant ($0.05 < p < 0.10$)	p	Optimal Setting
Temperature	0.071	1			
Age * Temperature	0.059				

Table 5

Comparison of the significant ANOVA results of depletions 1 to 5 with depletion 5.

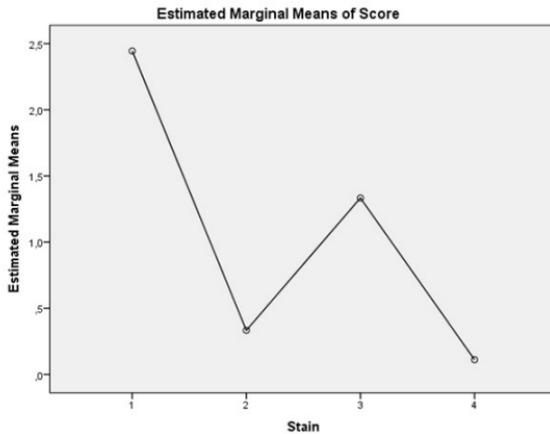


Figure 15

Main effect plot of the protein stains with which the impressions were enhanced. Stain 1 is AB, stain 2 is CS, stain 3 is CB, and stain 4 is HR. The Y axis represents the enhancement score of the impressions of depletion 5.

Conclusion

This research was conducted to further investigate the advantages and drawbacks of using alginate casts and with which protein stain the best results are achieved.

GC Aroma Fine Plus alginate lifted complete, clear impressions well, and the alginate was easy to handle and to chemically enhance.

The variables of age and protein stain had significant main effects. The highest levels of enhancement were achieved at age 2 (7 days) and when protein stain 1 (amido black) was used. Temperature was borderline significant, with the best level being 1 (8 °C). This means that optimal enhancement scores can be obtained from footwear impressions aged for 7 days in a cold environment, enhanced with amido black.

This research indicates that amido black is the preferred protein stain of use because it is the most sensitive protein stain of all four stains tested in this study and enhances impressions until the fifth depletion. Also, there are fewer health risks in using water-based AB than methanol-based CB. Therefore, it is safe to use at crime scenes for the recovery and detection of footwear impression evidence.

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