12.4 Diaminobenzidine

12.4.1 Introduction

Bloody footwear and tire tread impressions can be enhanced for the purpose of aiding forensic examinations. This procedure requires the use of a positive blood control and negative control prior to application.Enhanced impressions and/or substrates may experience additional color changes over time. It is recommended that the impression be photographed both before and immediately after processing.

12.4.2 Formula

Solution A (Fixative)

20 grams 5-Sulfosalicylic Acid

1 liter Distilled Water

In a 1 or 2 liter beaker on a magnetic stirring device dissolve the 5-Sulfosalicylic Acid in the distilled water.

Solution B (Buffer)

100 ml 1 M Phosphate Buffer (pH 7.4)

800 ml Distilled Water

In a 1 liter beaker on a magnetic stirring device, mix the Phosphate buffer with the distilled water.

Solution C (Developer)

1 gram Diaminobenzidine (DAB)

100 ml Distilled Water

In a 250 ml beaker on a magnetic stirring device, mix the DAB with the distilled water.

Working Solution

900 ml Solution B

100 ml Solution C

5 ml 30% Hydrogen Peroxide

Note: You may substitute 50 ml of 3% Hydrogen Peroxide for the 5 ml of 30% Hydrogen Peroxide.

12.4.3 Mixing

In a 2 liter beaker on a magnetic stirring device, mix 900 ml of Solution B with 100 ml of Solution C and add 5 ml of 30% Hydrogen Peroxide. This Working Solution should be mixed just prior to use. The Working Solution will be tested on a positive control blood stain prior to use. The quantities of the above solutions can be adjusted for processing large or numerous items.

12.4.4 Application

The solutions may be applied by dipping the specimen(s) to be enhanced in a container filled with the solutions or by covering the stained area with a paper towel(s) and using a squirt bottle filled with the solutions to saturate the towel(s).

Completely cover the target area. Remove the saturated paper towel(s) and apply a fresh one at each step in the procedure.

Immerse or saturate the stained area in Fixative (Solution A) for approximately 4 minutes. Remove and rinse well with distilled water. Immerse or saturate the stained area in the Working Solution and allow the color to develop for approximately three (3) to five (5) minutes or as long as the impression continues to darken. It will not harm development if the impressioned item is processed for a longer period of time. Remove and rinse in distilled water. Allow to air dry.

12.4.5 Reaction

Successful staining of the impression will result in a dark brown colored impression.

12.4.6 Storage

Solutions A and B can be stored indefinitely in amber bottles. Solution C can be stored frozen in an amber bottle for up to six months. The Working Solution must be mixed just prior to use.

12.4.7 References

Trozzi, T.A., "Developing Bloody Footwear Impressions with the Use of Diaminobenzidine", presented at the International Symposium on the Forensic Aspects of Footwear and Tire Impression Evidence, FBI Academy, Quantico, VA, 1994.

Bodziak, W. J., Footwear Impression Evidence, 2nd ed.; CRC Press: Boca Raton, FL, 2000.

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