SWGTREAD Scientific Working Group for Shoeprint and Tire Tread Evidence

Guide for the Chemical Enhancement of Bloody Footwear and Tire Impression Evidence (09/2008)

1. Scope

1.1 This Guide provides procedures for the chemical enhancement of bloody footwear and tire impression evidence in the field and in the laboratory. It is not intended to provide specific instructions or protocols for the collection, photography, and preservation of this or other evidence.

1.2 The particular procedures and methods employed in a given case will depend on the evidence.

1.3 This Guide may not cover all aspects of unusual or uncommon conditions.

1.4 This Guide does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this Guide to establish appropriate safety and health practices, be familiar with the Materials Safety Data Sheets (MSDS), and determine the applicability of regulatory limitations prior to use. Due to the inherent dangers associated with biohazard evidence, caution and appropriate measures should always be exercised when handling this type of evidence.

1.5 This Guide is not intended as a substitute for training in the chemical enhancement of bloody footwear and tire track evidence. Completion of a training program and experience in these skills is essential to understanding and applying the principles outlined in this Guide.

1.6 There are other chemical enhancement formulas and procedures for bloody impressions that are not listed in this Guide.

2. Terminology

Refer to the *Standard for Terminology Used for Forensic Footwear and Tire Impression Evidence* for a definition of terms used in this document.

3. Significance and Use

3.1 The procedures outlined here are grounded in the generally accepted body of knowledge and experience in the chemical enhancement of bloody footwear and tire

impression evidence. By following these procedures, a forensic footwear and tire tread examiner can reliably detect and/or enhance impressions made of blood.

3.2 Bloody footwear and tire tread impressions are enhanced for the purpose of aiding forensic examinations.

4. Interferences

4.1 Footwear and tire impression evidence may have inherent limitations that can interfere with the procedures in this Guide. Limitations, when known, should be noted and recorded.

4.2 Limitations can be due to substrate features, quality and quantity of original impressions and methods of collection and enhancement.

5. Equipment and Requirements

5.1 Assorted glassware

5.2 Hotplate with stirring capability

5.3 Magnetic stirrers

5.4 Scale/balance

5.5 Weighing boats

5.6 Lab coats

5.7 Safety goggles

5.8 Mist and dust mask

5.9 Disposable gloves

5.10 Fume hood – when available

5.11 Spray and squirt bottles

5.12 Amber glass storage bottles

5.13 Trays or appropriate containers

5.14 Paper towels

5.15 Appropriate chemicals and solvents per procedure

5.16 Alternate light source and colored barrier filter goggles

5.17 Photographic equipment and filters

5.18 Known blood stains for testing enhancement solutions

Note: Many of these enhancement chemicals are available as premixed kits.

6. Procedures

Record the specific lot or batch number of the chemicals used in the preparation of the enhancement solutions according to applicable laboratory procedures. At minimum, the container with the resulting enhancement solution should be labeled with the name of the enhancement solution and the date of preparation.

It should be noted that all of these procedures require the use of a positive blood control and negative control prior to application.

Note: A positive blood control is a known bloodstain to which the enhancement solution is applied to confirm that it is working. Caution should be used to avoid cross contamination of the enhancement solution with the known bloodstain.

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.

6.1 Amido Black (Fischer 98)

6.1.1 Formula

- 500 ml Distilled Water
- 20 grams 5-Sulfosalicylic Acid
- 3 grams Naphthol Blue Black (Amido Black)
- 3 grams Sodium Carbonate
- 50 ml Formic Acid
- 50 ml Glacial Acetic Acid
- 12.5 ml Kodak Photo Flo 600 Solution

6.1.2 Mixing

In a 2 liter beaker on a magnetic stirring device combine the ingredients in the order that they are listed. Dilute the mixture to 1 liter using distilled water. Stir until the Amido Black is dissolved. While this mixture can be used immediately, the best results will be obtained if it is allowed to stand for several days prior to use. The solution will be tested on a positive control bloodstain prior to use.

6.1.3 Application

The Amido Black solution may be applied by dipping the specimen(s) to be enhanced in a container filled with the solution, or by covering the stained area with a paper towel(s) and using a squirt bottle filled with the solution to saturate the stained area. Completely cover the target area and allow to develop for a minimum of thirty (30) seconds. Three (3) to five (5) minutes are preferred for maximum enhancement. The specimen(s) should be rinsed with tap water and allowed to air dry.

Note: Prior to application, a small area of the background of the object or surface being enhanced should be stained with the solution. If the background retains a significant amount of the stain, the Amido Black solution may not be appropriate for enhancement of this item.

6.1.4 Reaction

Successful staining of the impression will result in a blue-black colored impression.

6.1.5 Storage

The Amido Black solution can be stored in dark bottles indefinitely.

6.2 LCV – Leucocrystal Violet

6.2.1 Formula

- 10 grams 5-Sulfosalicylic Acid
- 500 ml 3% Hydrogen Peroxide
- 3.7 grams Sodium Acetate
- 1 gram Leucocrystal Violet

6.2.2 Mixing

In a 1 liter beaker on a magnetic stirring device dissolve the 5-Sulfosalicylic Acid in 3% Hydrogen Peroxide. Add the Sodium Acetate and Leucocrystal Violet (LCV). If the LCV crystals are yellow instead of white, do not use them. This is an indication that the reagent is old and the resulting solution will not be effective. The LCV solution will be tested on a positive control blood stain prior to use.

6.2.3 Application

The LCV solution may be applied by spraying the item to be enhanced with an aerosol sprayer, applying the liquid with a squeeze bottle, or immersing the item in a container filled with the LCV solution. The color reaction should

occur within 30 seconds. The enhanced impression should be rinsed with tap water after enhancement and allowed to dry.

6.2.4 Reaction

Successful staining of the impression will result in a violet colored impression. This reagent is sensitive to ultraviolet light and, within several days, the entire application area will turn violet, particularly if the background could not be rinsed. Developed impressions should be photographed as soon as possible.

6.2.5 Storage

The LCV solution can be stored in dark bottles for up to 30 days.

6.3 DAB – Diaminobenzidine

6.3.1 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.

6.3.2 Formula – 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.

6.3.3 Formula – 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.

6.3.4 Formula – 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.

6.3.5 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 bloodstain prior to use. The quantities of the above solutions can be adjusted for processing large or numerous items.

6.3.6 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.

6.3.4 Reaction

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

6.3.5 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.

6.4 Acid Fuchsin (Hungarian Red)

6.4.1 Formula

- 20 grams 5-Sulfosalicylic Acid
- 2 grams Acid Fuchsin
- 1 liter Distilled Water

6.4.2 Mixing

In a 2 liter beaker on a magnetic stirring device, dissolve the 5-Sulfosalicylic Acid and Acid Fuchsin in the distilled water. The solution will be tested on a positive control bloodstain prior to use.

6.4.3 Application

The Acid Fuchsin solution may be applied by dipping the specimen to be enhanced in a container filled with the solution or by using a squirt bottle filled with the solution to saturate the stained area. Completely cover the target area and allow to develop for approximately one minute. The specimen(s) should be rinsed with tap water and allowed to air dry.

Note: Prior to application, a small area of the background of the object or surface being enhanced should be stained with the solution. If the background develops a significant color, the Acid Fuchsin solution may not be appropriate for enhancement of this item.

6.4.4 Reaction

Successful staining of the impression will result in a deep magenta colored impression.

6.4.5 Storage

The Acid Fuchsin solution can be stored in clear or dark bottles indefinitely.

6.5 Luminol – this reagent is best used only on multi-colored or dark surfaces where other reagents will not produce an impression with sufficient contrast.

6.5.1 Formula

- 0.1 gram Luminol
- 5 grams Sodium Carbonate
- 0.7 grams Sodium Perborate
- 100 ml Distilled Water

6.5.2 Mixing

In a 150 ml beaker on a magnetic stirring device, dissolve the Luminol and the Sodium Carbonate in the distilled water. Just prior to using the mixture, add the Sodium Perborate and mix thoroughly. The Luminol solution will be tested on a positive control bloodstain prior to use.

6.5.3 Application

The Luminol solution may be applied by spraying the item to be enhanced with a non-metallic aerosol sprayer. It must be applied in total darkness. This chemical does not have a fixative in it and the blood may run with continuous spraying.

6.5.4 Reaction

A positive reaction will produce a blue white chemiluminescence which appears almost immediately and fades rapidly. Special photographic procedures using black and white film with long exposure times must be used to record the reaction at the instant that it luminesces. Spraying can be repeated during photography if necessary to maintain luminescence.

6.5.5 Storage

The Luminol solution must be mixed immediately prior to use.

6.6 Fluorescein – This reagent is best used on multi-colored or dark horizontal surfaces where other reagents will not produce an enhanced impression with sufficient contrast.

6.6.1 Formula – Fluorescein Stock Solution

- 1 gram Fluorescein powder
- 100 ml 10% Sodium Hydroxide solution
- 10 grams Zinc powder (preferably mossy)

In a 250 ml beaker on a hot plate with a magnetic stirring device, dissolve the Fluorescein in the Sodium Hydroxide solution with heat and gentle stirring. Add the Zinc powder and bring to boiling, stirring continuously. Boil until the solution loses most of its color. Cool and decant the liquid (Solution A) off of the Zinc powder.

6.6.2 Formula – 10% Hydrogen Peroxide

- 100 ml 30% Hydrogen Peroxide
- 200 ml Distilled Water

In a 500 ml beaker with a magnetic stirring device, mix the Hydrogen Peroxide with the distilled water.

6.6.3 Formula – Fluorescein Working Solution

- 10 ml Fluorescein Stock Solution

• 190 ml Distilled Water

6.6.4 Mixing

In a 250 ml beaker with a magnetic stirring device, mix 10 ml of Fluorescein Stock Solution with 190 ml of distilled water.

This Working Solution should be mixed just prior to use. The Working Solution, followed by spraying with 10% hydrogen peroxide, will be tested on a positive control blood stain prior to use.

6.6.5 Application

The Fluorescein Working Solution should be applied by spraying the item to be enhanced, followed by spraying with the 10% Hydrogen Peroxide solution. This reagent does not have a fixative in it and the blood may run with excessive spraying. Allow to air dry.

6.6.6 Reaction

A positive reaction will produce a fluorescent impression that requires excitation with an alternate light source of approximately 445 nm and the use of orange goggles for visualization and an orange filter for photography.

6.6.5 Storage

The Fluorescein Working Solution must be mixed just prior to use.

6.7 Ninhydrin – The use of Ninhydrin for the enhancement of bloody impressions is limited to impressions on porous non-glossy paper items. A fume hood should be used when applying Ninhydrin solution.

6.7.1 Formula

• 5 grams Ninhydrin

• 30 ml Methanol

• 40 ml Isopropanol

• 930 ml Petroleum Ether

6.7.2 Mixing

In a 2 liter beaker on a magnetic stirring device dissolve the Ninhydrin crystals in the Methanol. Add the Isopropanol and then the Petroleum Ether. The Ninhydrin solution will be tested on a positive control bloodstain prior to use.

6.7.3 Application

The Ninhydrin solution can be applied by spraying or dipping. Once the solution has been applied, it should be allowed to air dry. After the evidence is dry, the development process may be accelerated through the application of heat and humidity. If a humidity chamber is available, set the humidity between 60% and 70%. If a humidity chamber is unavailable, steam from a steam iron may be used as a source of humidity.

6.7.4 Reaction

Successful staining of the impression will produce a dark purple colored impression.

6.7.5 Storage

The Ninhydrin solution can be stored in dark bottles for up to one year.

7. Report

7.1 Examinations conducted, procedures utilized, evidence photographed, and conclusions reached should be documented and may also appear in a report.

8. Bibliography

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