OSAC 2022-S-0032
Best Practice Recommendation for the Chemical Processing of Footwear and Tire Impression Evidence

Footwear and Tire Subcommittee
Physics and Pattern Interpretation Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

Draft OSAC Proposed Standard

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Footwear and Tire Subcommittee
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Best Practice Recommendation for the Chemical Processing of Footwear and Tire Impression Evidence

Keywords: *footwear, tire, impression, evidence, chemical processing*

Abstract: Footwear and tire impressions encountered at a crime scene or on physical evidence associated with a crime scene may benefit from chemical processing. A variety of chemical processing techniques and formulas are available to attempt to develop additional details and contrast in the impression evidence. Techniques and formulations selected for chemical processing are based on the impression matrix, substrate, and other variables.

Foreword

The Footwear & Tire Subcommittee of the Organization of Scientific Area Committees is dedicated to providing the forensic community with best practices regarding footwear and tire impression evidence. This document is intended for use by the forensic professional and outlines best practice recommendations for chemical processing procedures for footwear and tire impressions at crime scenes and in the forensic laboratory.

This document originated as a proposal by the Footwear & Tire Subcommittee of the Organization of Scientific Area Committees.

This is the original issue of this document.

Table of Contents

1 Scope

This document is a best practice recommendation for forensic professionals who are responsible for the collection and examination of footwear and/or tire impression evidence encountered at crime scenes or in the forensic laboratory. Transfer impressions are commonly made on a two dimensional surface by a footwear or tire as a result of coming in contact with and acquiring dust, residue, blood, mud, or other materials that the footwear or tire subsequently deposits or transfers to a substrate in the form of an impression. Following the recommendations in this document can result in developing additional detail and/or contrast in footwear and tire impression evidence. Chemical processing procedures that are commonly used in the forensic community are included. This document does not purport to cover all chemical processing techniques or formulations that are available. Deviations from this document may preclude enhancement of impressions. This document is not intended as a substitute for training in chemical processing procedures for footwear and tire impression evidence. Completion of a training program and experience is essential to understanding and applying the principles outlined in this document.

2 Terms and Definitions

Alginate: A natural polysaccharide commonly used for lifting impressions.

Amino acid: An organic compound containing amine (-NH_2) and carboxyl (-COOH) functional groups, along with a side chain (R group)

Chemical Processing: A method or means of chemically changing one or more chemical compounds or substances typically via a color reaction.

Chemiluminescence: The low-temperature emission of light during a chemical reaction.

Control: A known standard or preparation for checking or verifying a test reagent.

Dental stone: A generic gypsum product generally having a rating of 8,000 psi or higher, commonly used to cast footwear and tire impressions.

Electrostatic lifter: An instrument which uses an electrostatic charge to transfer dry origin impressions from a substrate to a film.

Enhancement: Improving the visibility of an impression through physical, photographic, digital, optical, or chemical means.

False Negative: A test result which incorrectly indicates that a particular condition or attribute is absent.

False Positive: An unintended reaction which incorrectly indicates that a particular condition or attribute is present.

Fluorescence: Luminescence caused by the absorption of radiation at one wavelength followed by nearly immediate re-radiation usually at a different wavelength and that ceases almost at once when the incident radiation stops.

Forensic Light Source: A filtered light source that may be fixed or tunable to a variety of spectral ranges.

Gelatin lifter: A commercial product with gelatin applied to a pliable backing used to lift impressions.

Hemoglobin: A protein of red blood cells that contains iron and carries oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs.

Latent impression: An impression not readily visible to the naked eye.

Matrix/matrices: Substance(s) that are deposited or removed due to the result of coming in contact with a shoe or tire.

Oxidize: To combine or become combined chemically with oxygen.

Peroxidase reagent: An enzyme that catalyzes the oxidation of a particular substrate by hydrogen peroxide.

Phenolphthalein: A colorless crystalline solid used as a chemical indicator to detect for the possible presence of hemoglobin.

Physical techniques: Processes used to enhance or collect impressions such as lifting and casting methods (e.g. gelatin lifts, dental stone casts, alginate molds).

Reagent: Substance (usually a mixture or combination of chemicals) used in a chemical reaction to detect, examine, or produce other substances.

Safety Data Sheet (SDS): A document that contains information on the potential health effects of exposure to chemicals, or other potentially dangerous substances, and on safe working procedures when handling chemical products.

Sebaceous: Relating to the oil or waxy matter originating from the sebaceous glands.

Substrate: The surface upon which an impression is deposited.

Transfer impression: An impression made on a two dimensional surface by a footwear or tire as a result of coming in contact with and acquiring dust, residue, blood, mud, or other materials that the footwear or tire subsequently deposits or transfers to a substrate in the form of an impression.

3 Recommendations

3.1 Introduction

3.1.1 Chemical processing can be used to develop additional details in impressions that are faint or latent (non-visible). Chemical processing can also provide additional contrast between the impression and the underlying substrate.

3.1.2 Optical, photographic, physical and digital techniques may be used in conjunction with chemical processing to further enhance impressions.

3.1.2.1 An appropriate sequence of applications should be evaluated prior to processing.

3.1.2.2 Optical, photographic, and digital techniques for visualization/enhancement should be attempted prior to the chemical processing and physical techniques.

3.1.2.3 Physical techniques can be used prior to, and after, chemical processing and may maximize the recovery of evidence.

3.1.3 Chemical processing methods may be used individually or in sequence in order to maximize the recovery of evidence.

3.1.4 Chemical processing may be used in a crime scene environment when an item of evidence cannot be removed from the scene.

3.1.5 Consideration should be given to the removal of the impression evidence from the crime scene to be chemically processed in a controlled laboratory environment. Examples could include cutting out sections of flooring or drywall. Processing in a laboratory setting may allow for better control of the process and for the use of a greater variety of techniques.

3.2 Evidence Assessment and Evaluation

3.2.1 No single methodology exists for the chemical processing of impression evidence on all surfaces under all conditions. The training and experience of the practitioner is crucial to ensure that the variables associated with the evidence are considered and evaluated prior to chemical processing.

3.2.2 Variables to be evaluated and considered prior to attempting chemical processing may include:

3.2.2.1 Substrate composition (e.g. texture, porosity)

3.2.2.2 Substrate color

3.2.2.3 Substrate orientation (e.g. horizontal or vertical surfaces)

3.2.2.4 Stain/deposit matrices of the impression

3.2.2.5 Environmental conditions and/or limitations

3.2.2.6 Subsequent testing requirements (e.g. deoxyribonucleic acid (DNA) analysis)

3.2.3 Chemical processing reagents are specific to the stain/deposit matrices that are to be enhanced. Impressions should be assessed prior to selecting the chemical processing reagents to determine the possible matrix. General categories of common matrices are:

3.2.3.1 Blood

3.2.3.2 Environmental/Particulate deposits (elements or ions within dirt, dust, water)

3.2.3.3 Organic contaminants (skin, sebaceous, amino acids)

3.2.4 Impressions that may require subsequent DNA testing (e.g. blood, skin, etc.) should be sampled prior to enhancement provided that this will not destroy any detail that may be needed for comparison. Chemical processing techniques should be reviewed prior to use to ensure they are compatible with subsequent DNA analysis but DNA analysis on samples collected after chemical processing may be possible. Depending upon the situation additional sterile techniques may be necessary to prevent DNA contamination.

3.2.5 Avoid techniques and chemical processing which may compromise other forensic analyses that may be required.

3.3 Safety

3.3.1 Personal protective equipment such as lab coats, disposable sleeves, coveralls, shoe covers, eye protection, face masks, and gloves should be appropriately worn when preparing and using reagents.

3.3.2 Mix, and if possible, use chemicals in well ventilated areas or a chemical fume hood.

3.3.3 It is recommended that only water-based reagents be used in the field due to safety issues (e.g. flammability) with solvent-based reagents.

3.3.4 Face masks, respirators with appropriate filters, and fume hoods are recommended when applying reagents (spraying, toweling, pooling) in the field or lab.

3.3.5 Refer to relevant chemical Safety Data Sheets (SDS) for further information and precautions.

3.4 Quality Control

3.4.1 Reagents should be prepared using clean glassware, equipment and containers. The preparation area should be clean and free of contaminants.

3.4.2 At a minimum, containers should be labeled with the reagent name, date of preparation, initials of preparer and expiration date (if applicable) and other information as required.

3.4.3 A reagent preparation log may be maintained with the formulation used for each reagent, the lot numbers of the chemicals used, the date created, and initials of who prepared the reagent. SDS documents may also be contained within this log.

3.4.4 Prior to application on evidence, reagents shall be tested on known control samples to demonstrate that they react as expected. Information of what control was used and the results observed should be recorded. In some cases the reagent may also need to be tested against a small portion of impression, or sample of the stain/deposit, so as to make sure that the expected reaction takes place. Caution should be used when working with previously prepared reagents as they may have a limited shelf life.

3.4.5 It is recommended that a non-evidential area of the substrate be tested with each reagent to evaluate potential processing limitations such as poor de-staining, degradation of the substrate, or if the particular substrate also reacts with the reagent. This is particularly important if a sequence of more than one processing technique will be applied.

3.4.6 Commercially prepared reagents are available and may be used. Follow the manufacturer's instructions for these products. It is recommended that all quality control measures mentioned above be followed.

3.5 Documentation

3.5.1 Footwear and tire impressions should be documented prior to, during, and after processing. At a minimum, documentation should include photography but can also include diagrams, sketches, video, and notes.

3.5.2 Any impressions that have the potential to be used for comparison purposes shall be photographed using proper techniques prior to enhancement, and after enhancement, to capture examination quality photographs.

3.6 Matrices

3.6.1 Blood: Blood is commonly encountered at crime scenes and enhancement reagents for blood typically cause a color reaction with the protein components, or the heme group in hemoglobin, which are found in blood. Considerations for impressions in blood include the following:

3.6.1.1 Presumptive testing using a blood reagent such as phenolphthalein can be done in order to determine whether or not an impression could be blood. Precautions should be made to ensure that there is no loss in detail for comparison and the stain/deposit is not consumed in sampling.

3.6.1.2 It is recommended that impressions in blood that may require subsequent DNA testing should be sampled prior to enhancement, provided that this will not destroy any detail that may be needed for comparison.

3.6.1.3 Physical techniques can also be used prior to and after chemical processing of the impressions in blood.

3.6.1.4 Blood should be completely dry prior to chemical enhancement.

3.6.1.5 Fainter impressions may offer more opportunity for clarity/improved contrast with chemical processing than impressions with heavy deposits.

3.6.1.6 In general, older stains may be more receptive to chemical processing than fresh stains. Stains which exhibit suspected clean-up with bleach may also yield improved results with chemical processing after a period of time so that the bleach has degraded to form salt and oxygen which does not interact with the reagent.

3.6.1.7 With the exception of luminol, impressions in blood must be dry or fixed to the substrate prior to or during any chemical enhancement. For impressions containing a lot of blood, it may be desirable to pre-fix the impressions before chemical enhancement even if the fixative is included in a particular solution. A wipe of blood on a piece of clear acetate as a control allows for both the fixing and enhancement properties of the reagent to be tested.

3.6.1.8 Sequencing of chemicals can be done in the following order; peroxidase reagent (e.g. luminol, leucocrystal violet (LCV)) followed by protein stain (e.g. amido black). Generally, the peroxidase reagents are more sensitive for blood than the protein stains.

3.6.1.9 Impressions in blood can be lifted (gelatin, dental stone, alginate) post-enhancement from a surface in order to provide better contrast.

3.6.1.10 Luminol and LCV are particularly useful for spray applications over large areas. Amido black and Acid Yellow 7 are generally limited to the localized development of impressions.

3.6.1.11 Prior to application on evidence, reagents shall be tested on known control samples to demonstrate that they react as expected. Information of what control was used and the results observed (color change) should be recorded. In some cases the reagent may also need to be tested against a small portion of impression, or sample of the stain/deposit, so as to make sure that the expected reaction takes place.

3.6.1.12 It is recommended that a non-evidential area of the substrate be tested with each reagent to evaluate potential processing limitations such as poor de-staining, degradation of the substrate, or if the particular substrate also reacts with the reagent. This is particularly important if a sequence of more than one processing technique will be applied.

3.6.1.13 Even though unintended reactions can occur they can be useful in enhancing questioned impressions. For example, proteinaceous materials such as egg albumin will be enhanced with amido black.

3.6.1.14 None of the enhancement reagents are specific to human blood and will react with animal blood as well.

3.6.2 Environmental/Particulate deposits: Dust, dirt, or particulate impressions are commonly encountered at a scene of a crime. Sometimes the material which has been deposited may react with enhancement reagents based upon the reaction with the elements such as iron or calcium and ions such as carbonate. Considerations for impressions made in these deposits include the following:

3.6.2.1 Physical techniques can be used prior to, and after, chemical processing and may maximize the recovery of evidence. For example, an electrostatic lifter can be used first to lift dry residue impressions.

3.6.2.2 Faint impressions offer more opportunity for clarity/improved contrast with chemical processing than impressions with heavy deposits.

3.6.2.3 Enhanced impressions can be lifted (gelatin, dental stone, alginate) post-enhancement from a surface in order to provide better contrast.

3.6.2.4 Prior to application on evidence, reagents shall be tested on known control samples to demonstrate that they react as expected. Information of what control was used and the results observed (color change) should be recorded. In some cases the reagent may also need to be tested against a small portion of impression, or sample of the stain/deposit, so as to make sure that the expected reaction takes place.

3.6.2.5 It is recommended that a non-evidential area of the substrate be tested with each reagent to evaluate potential processing limitations such as poor de-staining, degradation of the substrate, or if the particular substrate also reacts with the reagent. This is particularly important if a sequence of more than one processing technique will be applied.

3.6.2.6 False positive reactions may occur with all of the enhancement reagents.

3.6.3 Organic components: There may be instances in which skin secretions are the matrix which gets deposited as an impression. This may have more use in enhancing impressions on clothing which may also involve blood but where other blood enhancements are inadequate, or are not successful, for the matrix. Considerations for impressions made in these deposits include the following:

3.6.3.1 One must consider if the deposit may require subsequent DNA testing. If so, a portion of the deposit should be sampled prior to enhancement provided that this will not destroy any detail that may be needed for comparison.

3.6.3.2 Prior to application on evidence, reagents shall be tested on known control samples to demonstrate that they react as expected. Information of what control was used and the results observed (color change) should be recorded. In some cases the reagent may also need to be tested against a small portion of impression, or sample of the stain/deposit, so as to make sure that the expected reaction takes place.

3.6.3.3 It is recommended that a non-evidential area of the substrate be tested with each reagent to evaluate potential processing limitations such as poor de-staining, degradation of the substrate, or if the particular substrate also reacts with the reagent. This is particularly important if a sequence of more than one processing technique will be applied.

3.6.3.4 False positive reactions may occur with all of the enhancement reagents.

3.7 Equipment

3.7.1 Spatula

3.7.2 Scale ("L" scales and straight scales)

3.7.3 Spray bottles (fine mist)

3.7.4 Stirring device

3.7.5 Graduated cylinders

3.7.6 Erlenmeyer flasks

3.7.7 Clear and/or dark storage bottles

3.7.8 Paper towel

3.7.9 Tongs

3.7.10 Glass trays

3.7.11 Disposable pipettes

3.7.12 Rinse bottle

3.7.13 Chalk

3.7.14 Forensic light source and appropriate goggles/glasses

3.7.15 Camera and accessories (refer to the document entitled "Best Practice Recommendation for Photographic Documentation of Footwear and Tire Impression Evidence" for further guidance)

3.7.16 Camera filters

335 3.7.17 Personal protective equipment

336 **3.8 Application Methods**

337 Chemical processing reagents may be applied through different methods. The general application
338 methods are described below. Refer to the individual chemical processing formulations in Annex A
339 of this document for specific application guidance.

340 3.8.1 Spraying

341 3.8.1.1 Use a fine mist sprayer to spray the chemical processing reagents onto the area to
342 be developed or fixed.

343 3.8.1.2 Pump or garden sprayers that dispense a larger volume of liquid or large droplets
344 are not recommended.

345 3.8.1.3 The use of sprayers can leave artifacts on the impression so the process should be
346 monitored closely during application.

347 3.8.2 Toweling

348 3.8.2.1 Place a piece of paper towel over the area to be developed or fixed and apply the
349 chemical processing reagents with a spray bottle or mist sprayer.

350 3.8.2.2 Do not use paper towels containing additives such as lotions or perfumes. Paper
351 towels with textured patterns should also be avoided as they may interfere with
352 the development process. Paper towels must also be sturdy enough not to
353 degrade during processing.

354 3.8.2.3 Air pockets may be removed using a roller to assure that all areas of the
355 impression are treated.

356 3.8.2.4 Leave the wet towel in place until development or fixation is complete. Remove
357 the towel and rinse the impression with distilled water or suitable rinse solution
358 as described for each chemical processing formulation.

359 3.8.3 Immersion

360 3.8.3.1 This application method may be used for items containing impressions that are
361 relatively small and mobile.

362 3.8.3.2 Place the item containing the impression into a tray of the chemical processing
363 reagent and leave it in place until development or fixation is complete.

364 3.8.3.3 Remove the item and rinse with distilled water or suitable rinse solution as
365 described for each chemical processing formulation.

366 3.8.4 Pooling

367 3.8.4.1 This application method may be used for items that are too large to move or are
368 otherwise immobile such as flooring, walls, or cabinets.

369 3.8.4.2 Apply the chemical processing reagents to the item containing the impression
370 using a disposable pipette, squeeze bottle, or other container.

371 3.8.4.3 Leave the reagents in place until development or fixation is complete.

372 3.8.4.4 Gently remove the excess reagent using a paper towel.

373

Annex A
(Formulations)

2% Sulfosalicylic Acid (2 % SSA) Fixative

A.1 Background

Blood is water soluble. A 2% solution of SSA is used to fix an impression in blood through the denaturing of proteins to the underlying substrate, prior to the application of aqueous-based reagents. This ensures that the impression is not dissolved or washed away during processing.

Do not use the fixative prior to the application of luminol as it will inhibit the chemiluminescence. Some reagent formulations may contain SSA (e.g. leucocrystal violet (LCV)). SSA should be used prior to enhancement with amido black and Acid Yellow 7.

A.2 Formulation

Combine 20 grams of 5-sulfosalicylic acid and 1 liter of distilled water to make a 2% solution.

Store in a dark bottle at room temperature.

Indefinite shelf life.

A.3 Quality Control

A wipe of blood on an acetate sheet can be used as a control to test the fixative properties. Leave fixative on the surface for 3-5 minutes and then rinse with water. Observe that no loss of detail is present.

A.4 Procedure

Apply using fine mist sprayer or through the immersion, pooling or toweling techniques. Leave on the impression for 3-5 minutes and carefully remove any excess solution using a clean paper towel.

A.5 References

Hussain, J. I.; Pounds, C. A., “The Enhancement of Marks in Blood, Part I, 5-Sulphosalicyclic acid: A Convenient and Effective Fixative for Marks Made in Blood”, CRE Report No. 649, Feb 1988.

Acid Yellow 7

A.1 Background

Acid Yellow 7 is a dye solution that is used for staining impressions made in blood. These impressions are stained yellow after treatment with Acid Yellow 7 and then fluoresce under blue/blue-green light. This technique is used to develop bloody latent impressions on dark, non-porous surfaces.

A.2 Formulation

Staining Solution:

1 g Acid Yellow 7

40 mL glacial acetic acid

250 mL ethanol

700 mL distilled water

Fixative Solution:

As the above reagent does not have a fixative one must use a fixative reagent such as 2% SSA.

Rinsing Solution:

40 mL glacial acetic acid

250 mL ethanol

700 mL distilled water

A.3 Quality Control

Deposit known blood control onto white substrate or medium of choice and spray with Acid Yellow. A positive test will result in fluorescence when viewed with a forensic light source in the 400nm-495nm range using a yellow or orange filter.

A.4 Procedure

Fix the impression with the Fixative Solution (2% sulfosalicylic acid (2% SSA)) and rinse with distilled water. Stain a small area of the evidence (separate from the impression) to check for background staining. If background staining occurs and will not rinse away with water, use a different enhancement method.

Spray the area with the staining solution and leave the stain to be in contact with the impression area for approximately 5 minutes.

Rinse thoroughly with rinse solution and allow to dry.

Observe the impression area using a forensic light source in the 400nm-495nm range using a yellow or orange filter.

The remaining blood may be further collected using a gelatin lifter.

A.5 References

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, *Journal of Forensic Identification*, 55(6): 741-763, 2005.

Amido Black – One Step (Water-Based)

A.1 Background

This enhancement procedure uses a water soluble dye that reacts with the protein in blood that produces a dark blue-black color in areas where blood is present. This amido black water-based formula is a one-step process which eliminates the need for a separate fix solution as it is incorporated into this formula. The amido black method can be used after treatment with leucocrystal violet (LCV) enhancement to further increase contrast.

A.2 Formulation

Using a stirring device, combine the following ingredients in the order that they are listed.

500 mL Distilled water

20 g 5-Sulfosalicylic acid

3 g Amido black (also known as amido 10B or naphthalene black)

3 g Sodium carbonate

50 mL Formic acid

50 mL Acetic acid

12.5 mL Kodak Photo-Flo 600 solution

Dilute this mixture to one liter using distilled water. For best results allow the mixture to stand (if possible) for several days prior to use.

A.3 Quality Control

Test the reagent with a known blood control. A positive reaction is a dark blue-black color.

A.4 Procedure

Using the amido black reagent, stain a small area of the evidence that is separate from the impression to check for background staining. If background staining occurs and will not rinse away with water, use a different enhancement method.

Apply the reagent to the area by either dipping, using a rinse bottle or apply using a fine mist. Completely cover the area in question and allow the area to develop for approximately 2 – 5 minutes. Once developed, rinse the area with distilled water.

A.5 References

Bodziak, W.J., *Forensic Footwear Evidence*. CRC Press: Boca Raton, FL: CRC Press; 2017.

Amido Black (Methanol-Based)

A.1 Background

This enhancement procedure uses a water soluble dye that reacts with the protein in blood that produces a dark blue-black color in areas where blood is present. This amido black methanol-based formula is a three-step process which requires the need for a separate fixative solution. The amido black method can be used after treatment with leucocrystal violet (LCV) enhancement to further increase contrast. Amido black is best used on nonporous substrates and whose background does not absorb the stain.

A.2 Formulation

Fixative Solution:

20 g 5-Sulfosalicylic acid

1000 mL Distilled water

Thoroughly dissolve the 5-sulfosalicylic acid in water.

Staining Solution:

900 mL Methanol

100 mL Glacial acetic acid

2 g Amido black (also known as amido 10B or naphthalene black)

Thoroughly dissolve the amido black in the acid/methanol solution.

Rinsing Solution:

900 mL Methanol

100 mL Glacial acetic acid

A.3 Quality Control

Test the reagent solutions with a known blood control. A positive reaction is a dark blue-black color.

A.4 Procedure

Fix the impression with the Fixative Solution and rinse with distilled water. Stain a small area of the evidence (separate from the impression) to check for background staining. If background staining occurs and will not rinse away with prepared rinsing solution, use a different enhancement method.

Apply the staining reagent to the area by either dipping, using a rinse bottle or apply using a fine mist. Completely cover the area in question and allow the area to develop for approximately 2 – 5

minutes. Once developed, use the rinsing solution and allow the area to dry. This step should not be eliminated as it helps to remove the stain from the background.

A.5 References

Bodziak, W.J., *Forensic Footwear Evidence*. CRC Press: Boca Raton, FL: CRC Press; 2017.

Barnett, K. G., Bone, R. G., Hall, P. W. and Ide, R. H. (1988) *The Use of Water Soluble Protein Dye for the Enhancement of Footwear Impressions in Blood on Non-Porous surfaces Part 1*, Forensic Science Service UK, Technical Note No. 629, July. London: Home Office.

Sears, V. G. and Prizeman, T. M. (2000) 'The Enhancement of Fingerprints in Blood – Part 1: The Optimization of Amido Black', *Journal of Forensic Identification*, vol. 50 (5), p 470.

Ammonium and Potassium Thiocyanate

A.1 Background

The thiocyanate ion, in an acid environment, will react with iron ions. Since iron is frequently found in soil and fertilizers, this method is a good choice for dirt or dust impressions.

A.2 Formulation

Potassium Thiocyanate:

Mix 15 ml of water with 120 ml of acetone.

Add 15 g of potassium thiocyanate.

Slowly add 10 ml of dilute sulfuric acid (1 ml of concentrated sulfuric acid to 9 ml of water) to the above mixture.

Always add the sulfuric acid to the acetone/water mixture. Do not add the acetone/water mixture to the acid or it may explode.

A milky mixture will result which will separate on standing. When the layers have separated, the top (clear) layer is removed and transferred to a glass bottle or spray unit. This is the working solution and is best if used immediately.

Ammonium Thiocyanate:

Mix 2g of ammonium thiocyanate in 90 mL of acetone.

Add 10ml of dilute nitric acid to the ammonium thiocyanate/acetone mixture.

Always add the nitric acid to the ammonium thiocyanate/acetone mixture. Do not add the ammonium thiocyanate/acetone mixture to the acid or it may explode.

No precipitation will result; no separation is required as with potassium thiocyanate.

A.3 Quality Control

The reagent is checked by using ferric chloride (or a comparable iron standard). A positive reaction will result in a red/brown color.

A.4 Procedure

It is best to check the thiocyanate solutions with the material which makes up the impression. A portion of this material is removed (if possible) and sprayed. If there is only a small amount of material which makes up the impression (and removal could disturb the impression) then a portion of the impression is isolated by a physical barrier and sprayed. A positive reaction will result in a red/brown color.

If no positive reaction occurs, the thiocyanate enhancement should not be done.

The solution is lightly sprayed (fine mist) and the amount of spraying should be controlled to get the maximum reaction without causing the impression to run or bleed.

If the reagent is not used immediately, it is best to be stored in a dark glass bottle.

A.5 References

Bodziak, W.J., *Forensic Footwear Evidence*. CRC Press: Boca Raton, FL: CRC Press; 2017.

Bodziak, W.J. *Footwear Impression Evidence: Detection, Recovery, and Examination*. 2nd Ed. Boca Raton, FL: CRC Press; 2000.

Froude, J.H. Using Ammonium Thiocyanate and Potassium Thiocyanate, *Journal of Forensic Identification*, 48:718 -724, 1998.

Bromophenol Blue

A.1 Background

Bromophenol, a pH indicator, can be used to enhance impressions in dust by reacting with calcium carbonate.

A.2 Formulation

Combine 20 grams of bromophenol blue and 1 liter of distilled water to make a 2% solution.

A.3 Quality Control

The reagent is checked by using calcium carbonate. A positive reaction will be a color change to blue.

A.4 Procedure

It is best to check the bromophenol blue solution with the material which makes up the impression. A portion of this material is removed (if possible) and sprayed. If there is only a small amount of material which makes up the impression (and removal could disturb the impression) then a portion of the impression is isolated by a physical barrier and sprayed. A positive reaction will result in a blue color.

If no positive reaction occurs, the enhancement should not be done.

The solution is lightly sprayed (fine mist) and the amount of spraying should be controlled to get the maximum reaction without causing the impression to run or bleed. If a reaction occurs but the color is yellow rather than blue, lightly spray water on the impression which should cause the impression to turn blue.

A.5 References

Glattstein, B., Shor, Y., Levin, N., Zeichner, A., pH Indicators as Chemical Reagents for the Enhancement of Footwear Marks, *Journal of Forensic Sciences*, 41 (1) (1996) 23-26.

McNeil, K., Knaap, W. Bromophenol Blue as a Chemical Enhancement Technique for Shoeprints. *Journal of Forensic Identification,* vol. 62(2), p. 143-153, 2012.

1,8-Diazafluoren-9-one (DFO)

A.1 Background

DFO is an amino acid reagent with fluorescent properties and can be used on porous surfaces which includes gel lifts.

A.2 Formulation

DFO stock solution:

1 g DFO crystals

200 mL Methanol

200 mL Ethyl Acetate

40 mL Glacial Acetic acid

Combine and stir with a magnetic stirrer until all ingredients are dissolved.

DFO working solution:

Add Petroleum ether to the stock solution until the total volume is 2 liters.

A.3 Quality Control

Place an amino acid rich deposit onto a porous surface and process with DFO. A positive test will fluoresce with the use of a laser or forensic light source.

A.4 Procedure

Submerge or spray the item for 5 seconds.

Air-dry the item in a fume hood.

Process the item a second time and air-dry the item in a fume hood.

Oven bake at 50 to 100 degrees C for 10 to 20 minutes.

View under a forensic light source at 495 nm to 550 nm. (Absorption Max is 514 nm. View under orange or red barrier filters.)

Image results using an orange colored or 550 (BP 35) bandpass filter.

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Hungarian Red

A.1 Background

Hungarian Red is a dye (Acid Fuchsin) solution in water/acetic acid mixture used for staining footwear impressions made in blood on non-porous surfaces.

A.2 Formulation

Fixative Solution:

2% Sulfosalicylic Acid Solution

5% Acetic Acid Washing Solution:

Add 10 mL of glacial acetic acid to 190 mL of distilled water in a large beaker.

Using a magnetic stir bar, stir the solution for 5 minutes.

Place the solution in a rinse bottle until needed.

Distilled water may be used in place of acetic acid solution.

Hungarian Red Working Solution:

Hungarian Red is available in a premixed solution and does not require prior mixing of this solution.

The solution should be placed in a rinse bottle to apply to an item of evidence.

A.3 Quality Control

Test the reagent with a known blood control. A positive reaction is a red color.

A.4 Procedure

Prior to spraying the item of evidence with any of the solutions, the bloody impression should be dried or cured to prevent the impression from dissolving when the solution is applied.

Cover the bloody impression with filter or tissue paper.

Spray the sulfosalicylic acid solution onto the tissue paper. Allow the tissue paper to remain on the item of evidence for two (2) minutes. For larger thick stains, the tissue should remain for a longer period of time.

Rinse the area of interest with distilled water.

Apply the Hungarian Red solution with a rinse bottle to the item of evidence ensuring the entire area is covered.

Wash the excess solution with the acetic acid solution (distilled water may be substituted in the step). Immediately blot any excess solution with the tissue paper.

Allow the item to dry (a hair dryer may be used to expedite the process).

When completely dry, place a white gelatin lifter over the impression. Leave the gelatin lifter on the impression for fifteen (15) to thirty (30) minutes.

Remove the gelatin lifter and view the lift with the laser or alternate light source. The most appropriate wavelengths are within the 515 to 560 nm range with a green filter and 600 nm with a red filter.

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http://bvda.com/en/hungarian-red

Leucocrystal Violet (LCV)

A.1 Background

Leucocrystal violet is the reduced or colorless form of crystal violet. When LCV and hydrogen peroxide come into contact with hemoglobin or its derivatives, a violet colored dye (crystal violet) is formed through the catalyzed oxidation from peroxide. This formulation includes a blood fixative, 5-sulfosalicylic acid. LCV is commonly used for application in large areas.

A.2 Formulation

Dissolve 10 grams of 5-sulfosalicylic acid in 500 mL of 3% hydrogen peroxide using a 500 mL bottle. (The 3% hydrogen peroxide sold in 473 mL bottles in stores also can be used.)

Add 4.4 grams of sodium acetate.

Add 1.1 grams of Leucocrystal violet.

If the LCV crystals are yellow instead of white, do not use. This means that the crystals are old and the solution may not be effective.

A.3 Quality Control

Test the reagent with a known blood control. A positive reaction is a dark violet color.

A.4 Procedure

Using the LCV reagent, spray a small area of the evidence that is separate from the impression to check for background staining. If background staining occurs and will not rinse away with water, use a different enhancement method.

Apply the reagent to the area by spraying a fine mist, soaking the area or by cascading the LCV over the area's surface.

On non-porous surfaces, such as tile, and on porous surfaces, when possible, the area should be rinsed with water approximately 2 to 3 minutes after the reagent has been applied.

LCV fluoresces and can be viewed and/or photographed under various wavelengths of ultraviolet and infrared light.

This solution must be stored in an amber bottle as it is light sensitive. This solution may be refrigerated to extend its reactivity. The solution shelf life is 30 days.

Amido black can be used after LCV treatment to further increase contrast.

A.5 References

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Luminol

A.1 Background

Luminol is a chemical that reacts with the heme compounds found in blood to produce a blue-colored chemiluminescence visible in a darkened area. Luminol is also known to react in a similar manner with other oxidizing agents (e.g. bleach). Luminol may assist in crime scenes where blood has been cleaned up from a surface and is no longer visible to the naked eye. Luminol can readily detect old bloodstains and minute amounts of blood that have been diluted or cleaned up.

A.2 Formulation

Dissolve 0.1 gram of Luminol and 5 grams of sodium carbonate in 100 mL of water.

Add 0.7 gram of sodium perborate and mix thoroughly.

Use reagent immediately.

A.3 Quality Control

This reagent should be used in a dark environment. The reagent is checked by using a copper standard (a penny) or a known blood control. A positive reaction will result in chemiluminescence.

A.4 Procedure

The area where the luminol reagent will be used should be as dark as possible. Extinguish all light sources and, if necessary, cover windows with some kind of material to darken the area.

Spray a fine mist of the reagent solution in a sweeping motion over the area of interest. Avoid saturation of the area.

If a positive reaction of an impression is observed, additional misting may be necessary for photography, with care taken not to dilute the stain.

Consideration should be given to presumptive testing for the presence of blood (e.g. phenolphthalein) and preservation for further DNA testing.

The reagent is a one-time use reagent and should be mixed immediately prior to use.

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Ninhydrin

A.1 Background

Ninhydrin is an amino acid developing reagent applied by dipping, brushing, or spraying. Development is catalyzed by the addition of heat and humidity to obtain a Ruhemann's Purple dye complex. Ninhydrin may also be used as a blood enhancement technique.

A.2 Formulation

5 g ninhydrin crystals

30 mL methanol

40 mL 2-propanol

930 mL petroleum ether

A.3 Quality Control

Place an amino acid rich deposit onto a porous surface, process with Ninhydrin, and transfer into a heat/humidity chamber. A positive test will result in a purple color.

A.4 Procedure

Application of the Ninhydrin solution may be accomplished through spraying, brushing, or dipping. After treating the evidence with the Ninhydrin solution, allow it to dry at room temperature. A 24-hour development period is recommended. Subjecting the item to a combination of heat and humidity can accelerate the reaction.

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Annex B
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