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

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



OSAC Proposed Standard

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

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Foreword

The Footwear & Tire Subcommittee of the Organization of Scientific Area Committees for Forensic Science provides 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.

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 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.

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

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

1 Scope

This document is applicable for forensic professionals who are responsible for the collection or examination of footwear or tire impression evidence encountered at crime scenes or in the forensic laboratory. This document provides guidance on using chemical processing methods to develop additional detail or contrast in footwear and tire impression evidence to make it easier to document, examine, or compare. 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 the 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 are essential to understanding and applying the principles outlined in this document.

2 Terms and Definitions

2.1
alginate
A natural polysaccharide commonly used for lifting impressions.

2.2
amino acid
An organic compound that acts as a building block for proteins and is found in blood and other organic residues.

2.3
chemical processing
A method or means of chemically changing one or more chemical compounds or substances typically via a color reaction.

2.4
chemiluminescence
The emission of light is a result of a chemical reaction.

2.5
control
Material of an established origin that is used to evaluate the performance of a test or comparison

2.6
dental stone
A generic gypsum product generally has a compression strength rating of 8,000 psi or higher, commonly used to cast footwear and tire impressions.

2.7
electrostatic lifter
An instrument that uses an electrostatic charge to transfer dry origin impressions from a substrate to a lifting film.

2.8
enhancement
Improving the visibility of an impression through physical, photographic, digital, optical, or chemical means.

2.9
fluorescence
Luminescence is 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.

2.10
forensic light source
A filtered light source that may be fixed to a single wavelength or tunable to a variety of spectral ranges.

2.11
gelatin lifter
A commercial product with gelatin applied to a pliable backing used to lift impressions.

2.12
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.

2.13
latent impression
An impression not readily visible to the naked eye.

2.14
matrix/matrices
Substance(s) that are deposited or removed due to contact with a shoe or tire.

2.15
oxidation
A process in which a chemical substance changes because of the addition of oxygen.

2.16
peroxidase reagent
An enzyme that catalyzes the oxidation of a particular substrate by hydrogen peroxide.

2.17
phenolphthalein
A colorless crystalline solid that is used as a chemical indicator to detect the possible presence of hemoglobin.

2.18
physical techniques
Processes used to enhance or collect impressions such as lifting and casting methods (e.g. powder, gelatin lifts, dental stone casts, alginate molds).

2.19
reagent
Substance (usually a mixture or combination of chemicals) used in a chemical reaction to detect, examine, or produce other substances.

2.20
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.

2.21
sebaceous
Relating to the oil or waxy matter originating from the sebaceous glands.

2.22
substrate
The surface upon which an impression is deposited.

2.23
transfer impression
An impression made on a two-dimensional surface by 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, which are considered non-destructive to the impression, 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 removing 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 are 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, but are not limited to:

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

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 or limitations

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

3.2.3 Chemical processing reagents interact with 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 (e.g., elements or ions within dirt, dust, water)

3.2.3.3 Organic contaminants (e.g., skin, sebaceous oils, 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; however, 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 Techniques and chemical processes that may compromise other forensic analyses should be avoided when possible.

3.3 Safety

3.3.1 Personal and chemical processes that may compromise other forensic analyses should be avoided when possible.

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

3.3.3 Only water-based reagents should be used at crime scenes due to safety issues (e.g., flammability) with solvent-based reagents.

3.3.4 Face masks, respirators with appropriate filters, or fume hoods are recommended when applying reagents (e.g., spraying, toweling, pooling) at crime scenes or in the lab.

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

3.4 Quality Control

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

3.4.2 Containers shall 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 will be maintained with the formulation used for each reagent, the lot numbers of the chemicals used, the date created, and the initials of the person 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. Previously prepared reagents should be tested on the day of use. Information on 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 the impression or sample of the stain/deposit to ensure that the expected reaction takes place.

3.4.5 Each reagent should be tested on a non-evidential area of the substrate to evaluate potential processing limitations, such as poor de-staining, degradation of the substrate, or whether the particular substrate also reacts with the reagent. This is especially 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. All quality control measures mentioned above should be followed.

3.5 Documentation

3.5.1 Footwear and tire impressions should be documented prior to, during (as appropriate), and after chemical processing. Documentation should include photography (to include examination quality photographs). Documentation can also include diagrams, sketches, video, and notes.

3.6 Matrices

3.6.1 Blood: Blood is commonly encountered at crime scenes and enhancement reagents for blood typically bind to or react with the protein components or the heme group in hemoglobin, resulting in a color change. Considerations for impressions in blood include the following:

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

3.6.1.2 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 or fixed to the substrate prior to chemical enhancement.

3.6.1.5 Faint 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 that exhibit suspected clean-up with bleach may also yield improved results with chemical processing (such as luminol) after a period of time, as 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 should 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 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 if needed to improve contrast. Generally, peroxidase reagents, such as luminol or leucocrystal violet (LCV), are used first followed by protein stains, such as amido black.

3.6.1.9 Impressions in blood can be lifted (gelatin lifts, 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 on 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 the impression or sample of the stain/deposit to ensure that the expected reaction takes place.

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

3.6.1.13 Unintended reactions that provide improved contrast between the impression and substrate can still be beneficial.

3.6.1.14 None of the 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 crime scenes. Sometimes the matrix contains elements such as iron or calcium and ions such as carbonate, which may react with enhancement reagents. 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 may 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 lifts, 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 on 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, to ensure that the expected reaction takes place.

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

3.6.2.6 Unintended reactions that provide improved contrast between the impression and substrate can still be beneficial.

3.6.3 Skin secretions: There may be instances, such as impressions on clothing, in which skin secretions (e.g., sweat, oils) or skin cells are the matrix that gets deposited. Although blood may also be present, blood enhancements may be inadequate for these impressions. 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 on 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 the impression or sample of the stain/deposit to ensure that the expected reaction takes place.

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

3.6.3.4 Unintended reactions that provide improved contrast between the impression and substrate can still be beneficial.

3.7 Equipment List (Not Exhaustive)

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

3.7.2 Clear or dark storage bottles

3.7.3 Disposable pipettes

3.7.4 Erlenmeyer flasks

3.7.5 Forensic light source and appropriate goggles/glasses

3.7.6 Gelatin lifters

3.7.7 Glass or plastic trays

3.7.8 Graduated cylinders

3.7.9 Heat/humidity chamber

3.7.10 Paper towels, filter paper, or tissue paper (non-textured, no perfumes/lotions)

3.7.11 Personal protective equipment

3.7.12 Rinse bottles

3.7.13 Scales ("L" scales and straight scales)

3.7.14 Spatulas

3.7.15 Spray bottles (fine mist)

3.7.16 Stirring devices

3.7.17 Tongs

3.8 Application Methods

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

3.8.1 Spraying

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

3.8.1.2 Sprayers that cannot be adjusted or dispense a larger volume of liquid or large droplets are not recommended.

3.8.1.3 Sprayers can leave artifacts (e.g., droplets) on the impression, so the process should be monitored closely during application.

3.8.2 Toweling

3.8.2.1 Place a piece of paper towel, filter paper, or tissue paper over the area to be developed or fixed and apply the chemical processing reagent with a spray bottle, mist sprayer, or pipette.

3.8.2.2 Do not use paper towels, filter papers, or tissue papers containing additives such as lotions or perfumes. Textured patterns should also be avoided as they may impart patterns onto the impression being developed. Paper towels, filter papers, and tissue papers must also be sturdy enough not to degrade during processing.

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

3.8.2.4 Leave the wet towel in place until development or fixation is complete. Then, remove the towel and rinse the impression with the specified rinse solution, as described for each chemical processing formulation.

3.8.3 Immersion

3.8.3.1 This application method may be used for items that are relatively small and mobile such as an article of clothing or a flooring tile.

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

3.8.3.3 Remove the item and rinse with the specified rinse solution as described for each chemical processing formulation.

3.8.4 Pooling

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

3.8.4.2 Apply the chemical processing reagent to the item containing the impression using a disposable pipette, rinse bottle, or other container.

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

3.8.4.4 Gently remove the excess reagent using a paper towel.

Annex A: 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 to the underlying substrate through the denaturing of proteins, prior to chemical enhancement. 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)).

A.2 Formulation

20 g 5-sulfosalicylic acid
1 L distilled water

Combine to make a 2% solution.

Store in a dark bottle at room temperature.

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. Examine to determine if any loss of detail has occurred.

A.4 Procedure

Apply using a fine mist sprayer or through the toweling, immersion, or pooling methods. 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-Sulphosalicylic acid: A Convenient and Effective Fixative for Marks Made in Blood", *Central Research Establishment Report* No. 649, 1988.

Annex B: Acid Yellow 7

B.1 Background

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

B.2 Formulation

Fixative Solution:
As this reagent does not have a fixative, the impression must be fixed using 2% SSA prior to enhancement.

Staining Solution:
1 g acid yellow 7
50 mL glacial acetic acid
250 mL ethanol
700 mL distilled water
Rinsing Solution:
50 mL glacial acetic acid
250 mL ethanol
700 mL distilled water

B.3 Quality Control

Deposit known blood control onto a non-porous substrate and apply the acid yellow 7 staining solution. A positive test will result in fluorescence when viewed with a forensic light source in the 400 nm-495 nm range using a yellow or orange filter.

Stain a small area of the item of evidence (separate from the impression) to check for background staining or any damage to the substrate. If background staining occurs and will not rinse away with the rinsing solution, or if the substrate shows evidence of damage or degradation, use a different enhancement method.

B.4 Procedure

Apply the fixative solution (2% SSA) to the impression. Leave it on for 3-5 minutes, and then carefully remove any excess solution using a clean paper towel.

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

Rinse thoroughly with the rinsing solution and allow to dry.

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

A white gelatin lifter can be used to lift the enhanced impression, which may allow for better visualization. The gelatin lifter should stay on the impression for 30 minutes under an evenly distributed 10–20-pound weight.

B.5 References

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

Sears, Vaugn G., et al., "Enhancement of Fingerprints in Blood, Part 3: Reactive Techniques, Acid Yellow 7 and Process Sequences." *Journal of Forensic Identification*, vol. 55 no. 6, 2005, pp. 741-763.

Annex C: Amido Black (Water-Based)

C.1 Background

This enhancement procedure uses a water-soluble dye that binds with the protein in blood to produce a blue-black color in areas where blood is present. This water-based amido black formula is a one-step process, as the fixative is incorporated into the solution. Amido black can be used after treatment with leucocrystal violet (LCV) to further increase contrast. Amido black is best used on nonporous substrates that do not absorb the stain.

C.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
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 1 L using distilled water. For best results allow the mixture to stand (if possible) for several days prior to use.

C.3 Quality Control

Test the reagent with a known blood control. A positive test will result in a blue-black color.

Stain a small area of the item of evidence (separate from the impression) to check for background staining or any damage to the substrate. If background staining occurs and will not rinse away with distilled water, or if the substrate shows evidence of damage or degradation, use a different enhancement method.

C.4 Procedure

Amido black can be applied using any of the methods. Completely cover the area in question and allow it to develop for approximately 2-5 minutes. Once developed, rinse the area with distilled water.

C.5 References

Bodziak, William. J. *Forensic Footwear Evidence*. 1st Ed., CRC Press, 2017.

Annex D: Amido Black (Methanol-Based)

D.1 Background

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

D.2 Formulation

Fixative Solution:
As this reagent does not have a fixative, the impression must be fixed using 2% SSA prior to enhancement.

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

D.3 Quality Control

Test the reagent solutions with a known blood control. A positive test will result in a blue-black color.

Stain a small area of the item of evidence (separate from the impression) to check for background staining or any damage to the substrate. If background staining occurs and will not rinse away with the rinsing solution, or if the substrate shows evidence of damage or degradation, use a different enhancement method.

D.4 Procedure

Apply the Fixative Solution (2% SSA) and rinse with distilled water. Apply the staining reagent to the area by either immersing or spraying 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 then allow the area to dry. This step should not be eliminated as it helps to remove the stain from the background.

D.5 References

Barnett, K. G., et al. “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 1988.

Bodziak, William J., *Forensic Footwear Evidence*. 1st ed., CRC Press, 2017.

Sears, Vaughn G. and Tania M. Prizeman. “Enhancement of Fingerprints in Blood – Part 1: The Optimization of Amido Black.” *Journal of Forensic Identification*, vol. 50, no. 5, 2000, pp. 470 - 480.

Annex E: Ammonium and Potassium Thiocyanate

E.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 and can be used on all substrates.

E.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. It is best if used immediately.

Ammonium Thiocyanate:
Dissolve 2 g of ammonium thiocyanate in 90 mL of acetone.
Add 10 mL of concentrated 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.

E.3 Quality Control

The reagent is checked by using ferric chloride (or a comparable iron standard). A positive test will result in a red/brown color. If not, this enhancement should not proceed.

It is best to check the thiocyanate solutions with the matrix that makes up the impression. A portion of this matrix is removed (if possible) and sprayed. If there is only a small amount of the matrix that 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.

E.4 Procedure

The solution is lightly sprayed (fine mist), and the amount of spraying should be controlled to achieve the maximum reaction without causing the impression to run or bleed. This reagent is best used in a laboratory setting.

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

E.5 References

Bodziak, William J. *Forensic Footwear Evidence*. 1st ed., CRC Press, 2017.

Froude, John H., Jr. "Using Ammonium Thiocyanate and Potassium Thiocyanate." *Journal of Forensic Identification*, vol. 48, no. 6, 1998, pp. 718 -724.

Annex F: Bromophenol Blue

F.1 Background

Bromophenol blue is a reagent that reacts with calcium ions commonly found in soil and dust. It has been found to work well on porous and non-porous substrates.

F.2 Formulation

1 g bromophenol blue
95 mL methanol
mL distilled water
Combine to make a 1% solution.

F.3 Quality Control

The reagent is checked by using calcium carbonate. A positive test will result in a blue color. If no positive reaction occurs, this method should not be used.

It is best to check the bromophenol blue solution with the matrix which makes up the impression.

A portion of this matrix is removed (if possible) and sprayed. If only a small amount of the matrix makes up the impression (and removal could disturb it), then a portion of the impression is isolated by a physical barrier and sprayed. A positive reaction will result in a blue color.

F.4 Procedure

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.

F.5 References

Bodziak, William J. *Forensic Footwear Evidence*. 1st ed., CRC Press, 2017.

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Annex G: 1,8-Diazafluoren-9-one (DFO)

G.1 Background

DFO is an amino acid reagent that reacts with amino acids found in blood and other biological materials. DFO-developed impressions will fluoresce with the use of a forensic light source or laser. DFO is used on porous surfaces, including gel lifts. It is a recommended technique when processing patterned or multi-colored substrates. DFO can be used to develop impressions on clothing that were created by the skin coming into contact with the clothing through impact. DFO cannot be used after ninhydrin processing.

G.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 L.

G.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 forensic light source at 495 nm-550 nm with an orange or red barrier filter.

G.4 Procedure

Immerse 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.
Heat the item using an oven set at 100 C and 0% humidity for 20 minutes. If an oven isn't available, a dry iron can be used. The item should be monitored for impression development.

View under a forensic light source at 495 nm-550 nm with an orange or red barrier filter.

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Annex H: Hungarian Red

H.1 Background

Hungarian Red is a water-based dye solution (acid fuchsin) that reacts with proteins and can be used to stain impressions made in blood on non-porous surfaces.

H.2 Formulation

Fixative Solution
As this reagent does not have a fixative, the impression must be fixed using 2% SSA prior to enhancement.

Hungarian Red Solution
20 g 5-sulfosalicylic acid
2 g Acid Fuchsin
1 L distilled water

*5% Acetic Acid Rinsing Solution**
10 mL glacial acetic acid
190 mL distilled
Using a magnetic stir bar, stir the solution for 5 minutes.
*Distilled water may be used in place of acetic acid solution.

H.3 Quality Control

Test the reagent with a known blood control. A positive test will result in a deep magenta color. Stain a small area of the item of evidence (separate from the impression) to check for background staining or any damage to the substrate. If background staining occurs and will not rinse away with the rinsing solution, or if the substrate shows evidence of damage or degradation, use a different enhancement method.

H.4 Procedure

Apply the fixative solution (2% SSA) and rinse with distilled water.
Apply the Hungarian Red Solution to the item of evidence using the pooling, toweling, immersion, or spraying methods, ensuring the entire area is covered. The solution should remain on the evidence for one to five minutes.

Rinse the excess solution with the 5% Acetic Acid Rinsing solution or distilled water. Immediately blot any excess solution with a paper towel or tissue paper.

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

The impression can be examined using a forensic light source when completely dry.

If needed to improve contrast, place a white gelatin lifter over the impression. Leave the gelatin lifter on the impression for fifteen to thirty minutes. During this time, use a weight to apply pressure to ensure complete contact with the impression.

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

Due to the possibility that the impression can diffuse into the gel lifter, photography of the impression should be conducted when it is removed from the evidence.

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Annex I: Leucocrystal Violet (LCV)

I.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. This technique is used to develop bloody latent impressions on porous and non-porous surfaces.

I.2 Formulation

Dissolve 10 g of 5-sulfosalicylic acid in 500 mL of 3% hydrogen peroxide using a 500 mL bottle.
Add 4.4 g of sodium acetate.
Add 1.1 g of leucocrystal violet.

Do not use LCV crystals that are yellow instead of white. The discoloration indicates that the crystals are old, and the solution may not be effective.

I.3 Quality Control

Test the reagent with a known blood control. A positive test will result in a dark violet color.

Stain a small area of the item of evidence (separate from the impression) to check for background staining or any damage to the substrate. If background staining occurs and will not rinse away with distilled water, or if the substrate shows evidence of damage or degradation, use a different enhancement method.

I.4 Procedure

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

The area should be rinsed with distilled water on non-porous surfaces, such as tile, and on porous surfaces, when possible, approximately 2 to 3 minutes after the reagent has been applied. If no rinse is applied, all areas treated with LCV (blood and non-blood) will eventually turn purple and may, therefore, obscure visualization of the enhanced impression.

In addition to visual observation/photography with white light, LCV fluoresces and can be viewed 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.

Protein stains such as amido black and Hungarian red can be used after LCV treatment to increase contrast further.

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Annex J: Luminol

J.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. The chemiluminescence that occurs is beneficial for visualizing bloody impressions on dark or multicolored surfaces. Luminol may assist in crime scenes by detecting where blood has been cleaned up from a surface and is no longer visible to the naked eye, where blood has been diluted, and where blood has aged. Luminol is also known to react similarly to other oxidizing agents (e.g., bleach). Luminol can be used on porous and non-porous surfaces; however, because no fixative is present in the luminol solution and cannot be applied prior to enhancement, luminol is best used on porous substrates.

J.2 Formulation

Dissolve 0.1 g of luminol and 5 g of sodium carbonate in 100 mL water.
Add 0.7 g of sodium perborate and mix thoroughly.
Use the reagent immediately.

J.3 Quality Control

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

J.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 to darken the area.

Spray a fine mist of the reagent solution in a sweeping motion over the area of interest, trying to avoid saturation.

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

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

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Annex K: Ninhydrin

K.1 Background

Ninhydrin is an amino acid-developing reagent applied by immersing 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 and for the development of impressions on clothing created by the skin coming into contact with the clothing through impact. It is used on porous surfaces.

K.2 Formulation

5 g ninhydrin crystals
30 mL methanol
40 mL 2-propanol
930 mL petroleum ether

K.3 Quality Control

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

A positive test will result in a purple color.

K.4 Procedure

Application of the Ninhydrin solution may be accomplished through immersing or spraying.

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 (e.g., using a steam iron) can accelerate the reaction.

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