

2017

Evaluation of current methods for processing bloody fingerprints on non-porous substrates exposed to various contaminants

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**EVALUATION OF CURRENT METHODS FOR PROCESSING BLOODY
FINGERPRINTS ON NON-POROUS SUBSTRATES EXPOSED TO VARIOUS
CONTAMINANTS**

by

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B.S., Fairleigh Dickinson University, 2015

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2017

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ACKNOWLEDGMENTS

I would like to thank my thesis committee, Amy Brodeur, Deborah Kosiorek and Kevin Kosiorek, Boston University School of Medicine, and my family for their support. I would also like to thank my fellow graduate student, Brian Engelson, for sharing his resources and advice.

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ABSTRACT

Blood is a common type of medium with which patent fingerprints are deposited at crime scenes. Chemical enhancements are generally used on bloody fingerprints when some type of pattern is visible but ridge characteristics are not sufficiently defined to make the print suitable for comparison. Motor vehicles, which may be associated with crimes scenes or forensic investigations, can be exposed to a variety of contaminants from the environment, including mud, salt, pollen, dust and motor oil, as well as from the application of chemicals to protect the paint such as car wax and car polish. It is unknown if these contaminants in combination with the chemicals used to enhance bloody impressions or in combination with the blood itself, could impact the enhancement of bloody impressions found on vehicles. This study seeks to assess the effectiveness of a selection of blood enhancement methods in the presence of such contaminants. Three of the four enhancement chemicals that were tested, Amido Black, Hungarian Red, and Leucocrystal Violet, were determined to be similarly effective for the enhancement of bloody friction ridge patterns applied to the surfaces of contaminated glass and metal substrates.

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LIST OF ABBREVIATIONS

5-SSA	5-Sulfosalicylic Acid
ACE-V	Analysis, Comparison, Evaluation - Verification
ALS	Alternate Light Source
AY7	Acid Yellow 7
AB	Amido Black
CAST	Centre for Applied Science and Technology
DNA	Deoxyribonucleic Acid
LCV	Leucocrystal Violet
HR	Hungarian Red
SWGFAST	Scientific Working Group on Friction Ridge Analysis, Study and Technology
Z1	Zinc Solution 1
Z2	Zinc Solution 2

1. INTRODUCTION

1.1 Fingerprint Overview

The use of fingerprints as a source of personal identification has been around for thousands of years, the earliest of which was discovered in ancient China, and various applications have continued to evolve over the years.⁽¹⁾ The development of fingerprint science in Europe dates back to the 17th and 18th centuries. One of the most important discoveries about fingerprints for forensic purposes was made by William Herschel who demonstrated the persistence of fingerprints by printing himself periodically over a period of more than 50 years until his death in 1917. In 1880, Henry Faulds observed and noted that fingerprints could be classified and that ridge detail is unique. In 1892, Sir Francis Galton established a system for classifying fingerprints in his book *Finger Prints*. He categorized print patterns into three major pattern types: loops, arches, and whorls. This would later lead to a more detailed classification system that is still used today.^(2,3)

One of the first recorded cases in which fingerprints were used to solve a crime took place in Argentina in 1892 in a case where a mother had accused her suitor of murdering her children but in fact had committed the act herself. This was determined after the comparison of a bloody thumbprint found at the scene of was identified to the mother rather than her suitor. Using fingerprints as a means of criminal identification began in the United States (New York) in the early 1900's.⁽¹⁾ Fingerprints are now frequently used as evidence in criminal investigations due to the persistent and unique nature of the

impressions left behind by individuals. At present, scientists in the field of forensics are striving to make identifications more objective to diminish bias in any examinations.^(2, 4)

1.1.1 Development of Friction Ridge Skin

Friction ridge skin is composed of ridges and furrows that make up the pattern on the skin of volar surfaces of the body. Volar skin refers to the palmar and plantar surfaces of the hands and feet. The ridge details on these surfaces of the body have been determined to be persistent (not permanent) and unique. They are considered persistent rather than permanent because skin cells are continually shed with new skin cells rising to the surface about every 30 days. Also, significant trauma and damage to the skin could affect the regeneration of the ridges or form scars that impact the original pattern of the skin since the scars persist through the skin regeneration process.⁽⁵⁾ The main evolutionary purpose of friction ridges is to provide a textured surface on the body to aid in gripping and holding objects.

Primary ridges begin forming at about 10.5 weeks into gestation, and primary and secondary ridge formation (minutiae) tends to become permanently set by week 17.⁽⁶⁾

Both the fetal development and later regeneration of skin produce skin surfaces with unique and persistent natural patterns and minutiae that are used for individualization.⁽⁵⁾

The general pattern of the ridges is determined by multiple factors including the shape and size of the volar pads that are formed during embryonic development, the time it takes for primary ridge formation to begin after the volar pads have been formed, the

speed of development of the ridges and the bone morphology underneath the skin of the volar pads.⁽⁷⁾ An individual's genetic make-up may contribute somewhat to the sizes and basic shapes of the patterns of the ridges but for the most part the embryonic environment and underlying bones in the hand and feet are the major determinants for the pattern formed. Identical twins, who have the same genetic make-up have never been observed to have identical prints.⁽⁸⁾

The skin can be divided in to two layers: the epidermis, or outer layer of skin and the dermis, or lower layer of skin. When regenerating, the cells move upward simultaneously with surrounding cells. The basal cells do not migrate but remain firmly attached to the generating layer. The persistence of the friction ridge detail on the surface of the skin is due to this process.⁽⁹⁾

1.1.2 The Comparative Value of Fingerprints

A fingerprint consists of the friction ridge skin of the last joint on each finger on the palmer surface of the hand. Minutiae, or ridge characteristics of fingerprints, include bifurcations, ending ridges, dots, short ridges, enclosures, and sometimes trifurcations. The types and locations of these minutiae that are seen in an individual's print contribute to its uniqueness.⁽²⁾

According to David Ashbaugh, a Canadian police officer who is known for his extensive research on friction ridge identification and who introduced the Analysis, Comparison, Evaluation – Verification (ACE-V) methodology for fingerprint identification, the individuality of fingerprints rests on four premises: “1) friction ridges develop in their definitive form in fetuses; 2) friction ridges remain unchanged throughout life with the exception of permanent scars; 3) the friction ridge patterns and details are unique; 4) the ridge patterns vary within certain boundaries that allow the patterns to be classified.”⁽¹⁰⁾

Classification systems allow an analyst to narrow down the group of possibilities of the unknown print based on similarities in basic ridge structures.⁽³⁾ The unique feature of skin can be classified based on three levels of detail. The first level includes general appearances of the ridge paths, direction of flow of ridges, and locations of any unique identifying marks such as scars, creases, and wrinkles that contribute to the pattern. Second level details include the path and outlines of the unique features. Lastly, third level details refer to characteristics and textures on the ridges such as pores and shape.⁽⁵⁾ Examples of level 1, 2, and 3 details can be seen in Figure 1. It is important to note that fingerprint examination is a comparative science, meaning that a reference impression is needed to compare any unknown fingerprints to since no identification could be made without a comparison to a known print.

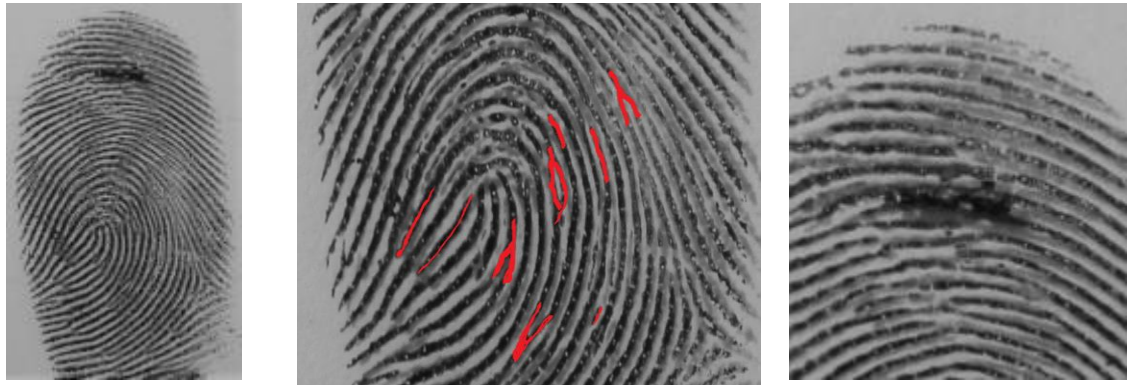


Figure 1. Three levels of detail found in friction ridge impressions. Left: Overall pattern (level 1); Center: Minutiae are highlighted in red (level 2); Right: Fine details of pores and ridges (level 3)

The purpose of developing and collecting fingerprints is for comparison to a known source, usually a suspect in a crime or to fingerprints in a pre-existing database. Level 1 cannot be used for individualization but can be used to exclude two prints from having a common source. Level 2 details include the unique minutiae that allow for individualization of a print based on their presence and location in the two prints being compared. Level three details include more fine details including shapes of ridge edges and locations of pores. This level requires really high quality photography of the print to be visualized so it is not required for individualization but rather it strengthens the argument for an inclusion if present.⁽²⁾

The ACE-V method was instituted by the Scientific Working Group on Friction Ridge Analysis, Study and Technology (SWGFAST) to standardize fingerprint examinations.⁽¹¹⁾ The first step, analysis, involves examining the unknown fingerprint evidence to determine visible ridges and clarity. In this step, features of the print are categorized and the print is determined to be either suitable or unsuitable for comparison. The second

step, comparison, involves the side by side comparison of known and unknown prints focusing on level 1, 2 and 3 details. The analyst will focus on features that were identified during the analysis stage to avoid bias. The third step, evaluation, refers to the inferences made about the identity of the source of unknown print. The three terms used in these determinations are: exclusion, individualization, or inconclusive. The final stage, Verification, involves the analysis, comparison, and evaluation of the same prints by another qualified examiner.⁽⁹⁾

Three main types of prints that can be found at a crime scene include patent, latent, or impressed/plastic prints. Latent prints are not usually visible to the human eye without the aid of an alternate light source and are composed of the natural components excreted from the volar surfaces of human skin, such as sweat and oils. These prints can be enhanced with powders and chemical enhancements. Impressed prints are those that have been pressed into a soft material that has retained the pattern of the skin. Casting and molding materials may be used to collect such prints. Patent prints can generally be visible to the human eye since the print is usually made in contaminants that have coated the surfaces of volar skin and transferred the pattern to a surface in that medium. Depending on the medium in which the print was transferred in, a variety of collection techniques can be used to collect and visualize these prints.^(3,4)

1.1.3 Impressions Made in Blood

Blood is one of the most common types of mediums with which patent fingerprints are deposited at crime scenes. Due to the adhesive nature of blood, once a small amount contaminates an object, that object can then contaminate others and thereby transfer a pattern.⁽¹²⁾ If the 'object' is the volar surface of a finger, then the ridge pattern can be transferred in blood to any surface it comes into contact with.

Bloody fingerprints are often encountered in cases of violent crime where an offender has caused harm to the victim or to themselves resulting in the presence of blood. When a bloody fingerprint is deposited at a crime scene in which the blood belongs to the victim but the fingerprint impression does not, that evidence becomes very powerful.⁽⁹⁾ This evidence can link the suspect to the scene of the crime at the time the crime took place. Latent fingermarks tend to repel blood so it is unlikely that a preexisting latent fingermark was developed by the blood to produce a bloody fingerprint at the scene.⁽¹³⁾ Therefore, the preservation and collection of such impressions becomes very important to the outcome of the investigation.

Chemical enhancements are generally used on bloody fingerprints when some type of pattern is visible but ridge characteristics are not sufficiently defined to make the print suitable for comparison. Most chemical enhancement methods for bloody fingerprints do not interfere with subsequent DNA testing. Some bloody fingerprint enhancement methods are based on the peroxidase-like activity of hemoglobin while others utilize

general protein staining dyes.⁽⁴⁾ The purpose of chemical enhancement methods is to make faint impressions more visible and to provide contrast between the print and the background substrate so that miniscule ridge details can be observed and interpreted more effectively.

In the mid 1800's researchers began developing presumptive tests for blood that would not require the destruction of physical evidence such as fingerprints and footwear impressions. In the 1900's the use of protein dyes such as Amido Black, Coomassie Blue, and Hungarian Red started to become popular with forensic investigators as methods to detect such prints.⁽¹⁾ The methanol formulation of these protein dyes encouraged a change in fixative from heat to submersion in methanol. Fixatives are applied before enhancement with dyes to alter the components of blood, allowing them to 'fix' or adhere to a surface better. This prevents the water-soluble bloody fingerprints from being dissolved or washed away by the aqueous chemical enhancements. Future formulation adjustments lead to aqueous solutions of the dyes and eventually the use of 5-Sulfosalicylic acid (5-SSA) as the fixative of choice. The aqueous solutions came into favor due to the safer nature of the solutions for the analysts working with them. Research on various other enhancement methods in addition to protein dyes continued throughout the end of the 1900's and in 1996, Leucocrystal Violet (LCV) was discovered as a presumptive enhancement method for blood by William Bodziak.⁽¹⁾

1.2 Current Processing Methods

Blood makes up about 8% of the total body weight of humans. It has an adhesive quality that can result in a variety of pattern transfers in the course of a crime. Plasma makes up about 55% of blood. Plasma is made up of mostly water but also contains proteins, organic acids, and salts. The other 45% of blood contains cellular material including red blood cells, white blood cells and platelets. The main protein found in red blood cells, hemoglobin, is one of the proteins used to presumptively determine the presence of blood. Hemoglobin is a protein that makes up about 95% of the protein volume in red blood cells and is responsible for the transport of oxygen throughout the body.^(4, 12)

Chemical blood enhancements can be categorized into two groups based on their method of action: those that react with the heme group and those that react with protein and various protein breakdown products.⁽¹⁾

1.1.2.1 Presumptive Chemical Enhancements: Leucocrystal Violet

The first group is considered presumptive for the presence of blood and consists of reagents that react with the heme group in blood to effect a color change. The reaction with the heme group usually involves a reduction-oxidation reaction. Hemoglobin is able to catalyze the reduction of hydrogen peroxide in the solution to water and oxygen. The colorless reduced dye is then oxidized to form a colored product.⁽¹⁾

Leucocrystal violet is a colorless solution that reacts with the hemoglobin in blood to create a purple colored product known as crystal violet (Figure 2).⁽¹⁴⁾ As it is an inherently colorless solution, it won't automatically stain the background substrate providing for optimal contrast and allows it to be used on porous surfaces. In addition, this solution also acts as an indicator of blood since the hemoglobin molecule in blood acts as a catalyst in order to effect a color change. In most instances, there will be no color change if the print was not made in blood.

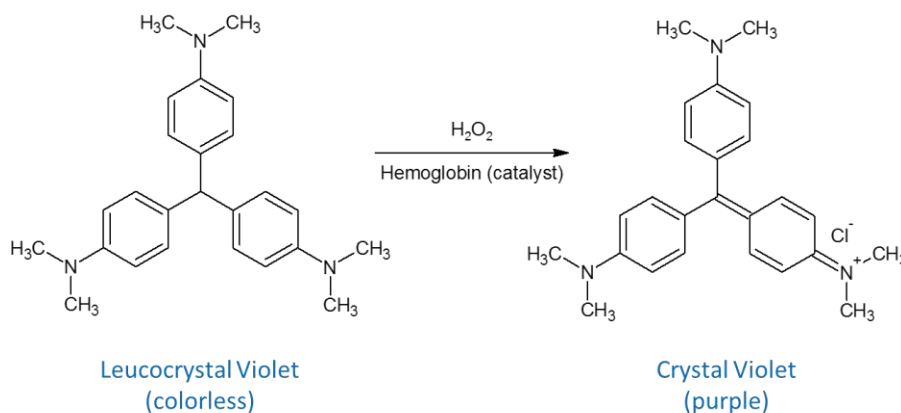


Figure 2. Chemical reaction of leucocrystal violet in the presence of blood.

1.1.2.2 Protein Dyes: Amido Black, Hungarian Red, and Acid Yellow 7

The second group, also known as protein dyes, contains enhancements that react with proteins or their breakdown products to effect a color change and are not specific to blood but tend to be relatively sensitive due to the high percentage of proteins in blood. The most effective types of protein dyes are acidic dyes which contain sulphonate ($-\text{SO}_3^-$) groups that allow the dyes to be soluble in either water or alcohol, the two main solvents used to make the dye solutions.⁽¹⁵⁾ It also aids in the reaction between the dye and the

blood. In acidic conditions the proteins in blood tend to become positively charged and are thus attracted to the negative charge of the sulphonate groups.⁽¹⁾ The chemical structures of the dyes used in this experiment are shown in Figure 3.

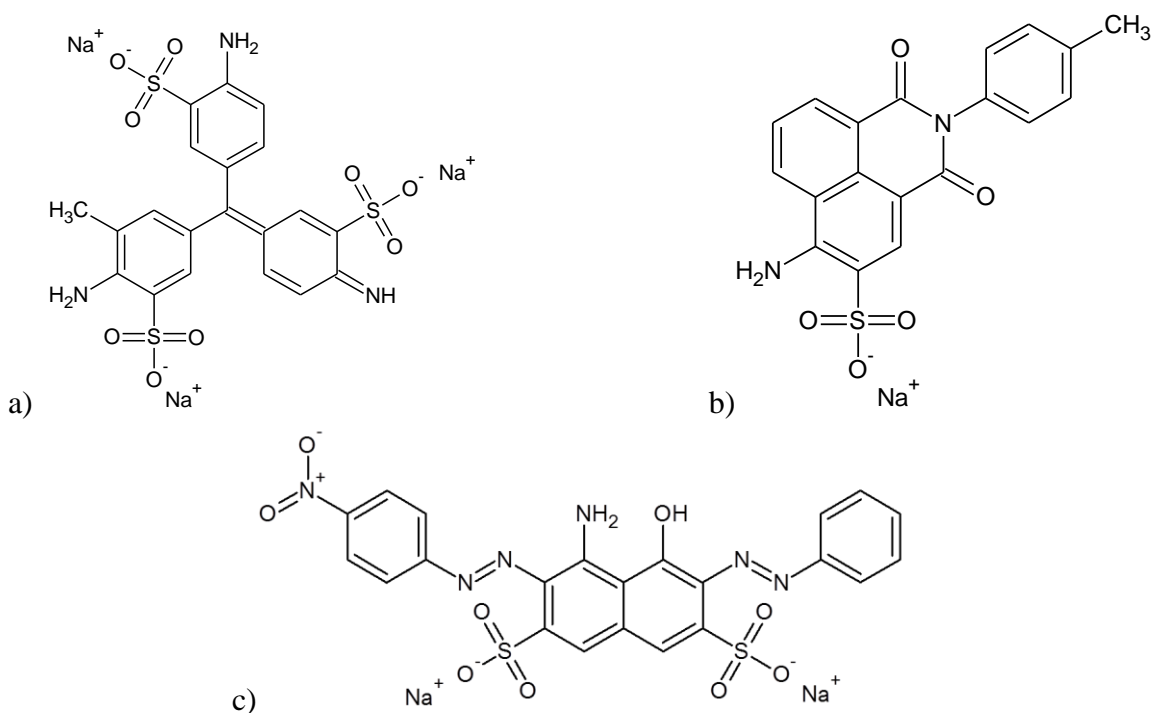


Figure 3. Chemical structures of three acid dyes used in the enhancement of blood impressions. a) Hungarian Red; b) Acid Yellow 7; and c) Amido Black.

Protein dyes differ from Leucocrystal Violet reagent in that they are not initially clear solutions that react with blood to form a color change. They are solutions that are composed of color pigments suspended in a solution that can be either aqueous or methanol based. Since they contain color pigments they are primarily used on non-porous surfaces due to the possibility of seeping into and staining porous items. The sulphonate groups in these dyes react with the proteins in blood in the presence of acid to form a color complex (Figure 4).⁽¹⁵⁾ The acid is usually found in the reagent solution and

is most commonly acetic acid. Once the pigments react with the blood proteins, the excess reagent can be washed away with water leaving behind a stained impression.

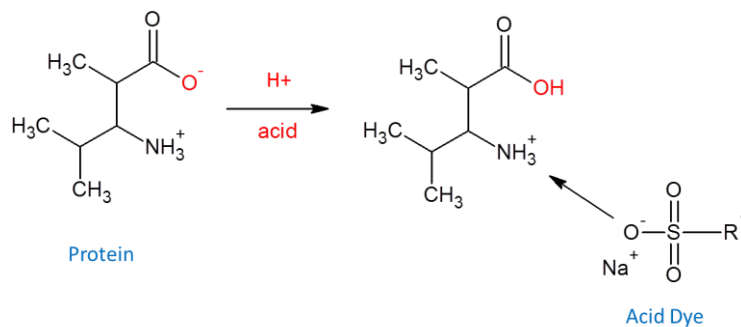


Figure 4. Reaction of acid dyes in the presence of proteins in in blood.

Amido Black (AB) aqueous solution, also known as Acid Black 1 or Naphthol Blue Black, is a dark blueish-black solution that stains the proteins in blood, other than hemoglobin, a bluish-gray color. Since it is not specific to blood, the solution may stain other substances containing proteins. It is safe to use both in the lab and at the crime scene.

Hungarian Red (HR) solution, also known as Acid Fuchsin or Acid Violet 19, is dark reddish-pink in color and also stains proteins found in blood, as well as other mediums, a red color. It has the ability to fluoresce using green light (515-560 nm) when lifted onto a white gelatin lift and a red filter is used to visualize the fluorescence.⁽¹⁶⁾ It is considered safe for both laboratory and crime scene use if proper personal protective equipment is utilized.

Acid Yellow 7 (AY7) solution is a vibrant yellow transparent liquid. In the presence of blood it will produce a fluorescent specimen that can be observed using blue light (420-485 nm) and an orange barrier filter. It tends to be less effective on heavier deposits of blood because the hemoglobin absorbs the emitted light, quenching the fluorescence.⁽¹⁾

1.1.2.3 Fixatives

Prior to enhancement of bloody impressions, the prints need to be fixed to the surface to prevent the print from being destroyed or damaged by the enhancement process. Since blood is an aqueous medium it will wash away in the presence of aqueous solutions, of which most chemical enhancements today are composed. Therefore, a fixative must be applied to the bloody impression in order to fix the impression in place. Natural aging of a blood stain causes denaturation, which precipitates the proteins in blood allowing them to adhere to surfaces and not be washed away as easily. Since this aging process occurs over a longer period of time chemical fixatives are applied to speed up the process.⁽¹⁷⁾ Throughout the years various fixatives have been used to secure blood spatter and blood impressions so that evidence could be preserved, many of which were determined to be carcinogenic and dangerous to the analysts.⁽¹⁸⁾ In 1988, Hussain and Pounds discovered that a 2% aqueous solution of 5-sulphosalicylic acid was both a safe and effective fixative for bloody impressions, and it has become the fixative of choice for bloody fingerprints that are going to be processed with aqueous based chemical enhancements.⁽¹⁹⁾ The chemical structure of 5-sulfosalicylic acid is shown in Figure 5.

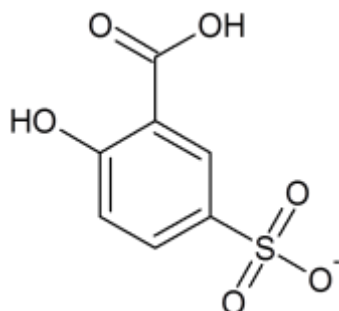


Figure 5. Chemical structure of 5-sulfosalicylic acid.

The 2% 5-sulphosalicylic acid solution is thought to precipitate the basic proteins in blood by either the formation of salt complexes or by the disruption of the protein structure but the exact mechanism is unknown.⁽¹⁵⁾ Its application ultimately leads to the impression being fixed in a short amount of time, usually in minutes, which allows investigators to enhance the prints quickly without damaging the structure and quality of the impression. Other fixatives may work to fix an impression by alternative mechanisms such as cross-linking, dehydrating and precipitating proteins. Methanol, which is used as the fixative when the solution has methanol as the major solvent, dehydrates blood resulting in the precipitation of proteins.⁽¹⁾

1.3 Difficulties with Processing on Challenging Surfaces

The enhancement methods explained above are usually performed on relatively clean and non-porous surfaces. Motor-vehicles, which are commonly used in the course of various crimes, can be exposed to a variety of contaminants from the environment, from splash up when driving, and from the application of chemicals to protect the paint coating the

car and to correct for scratches or imperfections in the coating. It is unknown if these contaminants in combination with the chemicals used to enhance bloody impressions or in combination with the blood itself, could impact the enhancement of bloody impressions found on vehicles. Anecdotal evidence suggests a difficulty in enhancing prints on a heavily contaminated car during the winter season (personal communication, D. Kosiorek, Boston Police Department). Specifically, it was believed that the layer of snowy, muddy slush kicked up from the road onto the surface of the vehicle prevented the successful enhancement of bloody prints that were initially visualized on top of this contamination in several cases.

This study is intended to assess if the current enhancement methods used are still effective when surface contaminants are present or if other methods should be considered. First, the effectiveness of the current fixative used to fix bloody fingerprints, 5-sulfosalicylic acid, is evaluated alongside methanol, two zinc fixative solutions, acetone, and heat. Methanol is currently used to fix bloody impressions that are enhanced with stains that contain methanol as a solvent. The two zinc solutions were chosen due to their non-hazardous nature and based on the conclusions of a study in which bloodstains fixed with the zinc solutions resulted in more crisp and defined patterns.⁽²⁰⁾ Heat fixing is a method used in histology and was the method used prior to the discovery of chemical fixatives. Acetone was chosen because it has been used as a fixative in histology with a similar method of action to alcohol fixatives, and it can be found in most laboratories.⁽²¹⁾

The contaminants evaluated in this study include environmental contaminants such as pollen and dust, contaminants that splash up from the road including mud, salty-snow mixtures, and motor oil, and contaminants that are applied to the surfaces of vehicles for aesthetic purposes such as car polish and car wax. Car wax and car polish are distinguished by their purpose. Car wax is applied to fill in any scratches or divots, while car polish usually contains some sort of abrasive in its formulation in order to buff away a thin layer of the surface of the paint to smooth out scratches or imperfections.⁽²²⁾ A variety of blood enhancement reagents are tested on glass and metal surfaces coated with the various contaminants to evaluate their effectiveness, as well.

2. MATERIALS AND METHODS

2.1 Application of Bloody Fingerprints to Various Surfaces

Bloody fingerprints were applied using refrigerated bovine blood that contained preservatives. The method used to deposit the bloody fingerprints was adapted from the study performed by David Petreti et al.⁽²³⁾ First, the refrigerated blood was brought to room temperature. A few drops of blood were then poured into a weigh boat containing a piece of paper towel. The right index finger, ungloved, was dipped into the blood soaked paper towel, then removed and left to air dry for approximately one second. The index finger and thumb on the same hand were then rubbed together to evenly coat the blood on the index finger. The finger pad containing the blood was left to air dry a few seconds more before depositing a series of three fingerprints. The blood was not replenished between the three impressions so as to make a diminishing series.

2.2 Application of Fixatives and Chemical Enhancements

The toweling method was used to apply both the liquid fixatives and the chemical enhancements to the bloody fingerprints. In the case of vehicles, it would be impossible to submerge a portion of the car without dismemberment to the car, and since many of the surfaces are vertical or curved, liquids tend to quickly cascade off the surface and reapplication of the reagent would need to be frequent. The toweling method was chosen because the towel allows the fixative/chemical enhancement to stay in contact with the bloody print for prolonged periods of time without having to submerge the item or continually reapply the chemical.

This method involves first applying a clean, pattern-less paper towel to the surface containing the prints to be enhanced. The fixative or reagent is then gently pooled onto the paper towel until moist and re-applied when the paper towel starts to absorb the liquid or starts to dry out. This process is continued for a fixed period of time, usually several minutes, and then the paper towel is carefully removed and the surface rinsed with either water or a designated rinse solution.⁽²⁴⁾

In this experiment the toweling method was used for both applying the fixative and applying the chemical enhancement to the surfaces containing the bloody fingerprints. Figure 6 shows the complete processing method used on each item containing bloody impressions. The fixative was applied for five minutes followed by a rinse with a few milliliters of water. The chemical enhancement was then applied for three minutes followed by a rinse with water, or a designated rinse solution in the case of Acid Yellow 7.⁽²⁴⁾



Figure 6. Flow chart of bloody fingerprint processing using the toweling method.

2.3 Evaluation of Various Fixatives

The purpose of the first part of this experiment was to evaluate the effectiveness of various fixatives in comparison to the current fixative used in bloody fingerprint processing, which is a 2% aqueous solution of 5-SSA. This fixative was compared to five other protein or blood fixatives found in the literature: heat, methanol, acetone, and

two zinc solutions. The first zinc fixative (Z1) was an aqueous solution that contained 0.05% calcium acetate, 0.5% zinc acetate, and 0.5% zinc chloride. The second zinc solution (Z2) was prepared in a similar way except it contained 0.5% zinc trifluoroacetate rather than zinc acetate.⁽²⁰⁾ The compositions of the various fixative solutions are shown in Table 1.

Table 1. List of fixative solutions.

Fixatives	Contents	Manufacturer
5-Sulfosalicylic Acid (2%)	20g 5-Sulfosalicylic Acid 1000 mL Distilled Water	ACROS Organics -
Zinc Solution 1	0.05g Calcium Acetate 0.5g Zinc Chloride 0.5g Zinc Acetate 100 mL Distilled Water	Alfa Aesar Fisher Alfa Aesar -
Zinc Solution 2	0.05g Calcium Acetate 0.5g Zinc Chloride 0.5g Zinc Trifluoroacetate 100 mL Distilled Water	Alfa Aesar Fisher Alfa Aesar -
Methanol	100% Methanol	Fisher
Acetone	100% Acetone	Fisher

Initially, a diminishing series of three bloody fingerprints was applied to twenty-four clean, labeled glass slides using the method previously described. The bloody prints were left to air-dry for at least 10 minutes before processing. The slides were then fixed using the six different fixatives, four slides per fixative. The liquid fixatives were applied using the toweling method; for the heat fixative, the surfaces containing the bloody prints were subjected to high heat from a hair dryer, held about 4-6 inches from the surface, for five minutes.

The four slides in each set were each tested using one of the four chemical enhancement methods: Leucocrystal Violet, Amido Black, Hungarian Red, and Acid Yellow 7. The different fixative methods were evaluated based on the presence or lack of presence of an enhanced fingerprint.

Next, six sets of three prints in a diminishing series were placed onto a painted metal sheet as shown in Figure 7. To prevent the heat from affecting the 5 other sets of prints, one set of bloody prints was applied to the ‘heat’ section of the metal piece, and these prints were heat fixed before the other prints were applied upon the metal returning to room temperature. Each of the other five sets of prints was fixed one at a time using a different fixative. The metal sheet was tilted, either left or right depending on the location of the section, during fixing to prevent contamination of the other sections. This process was repeated for each of the four chemical enhancements.

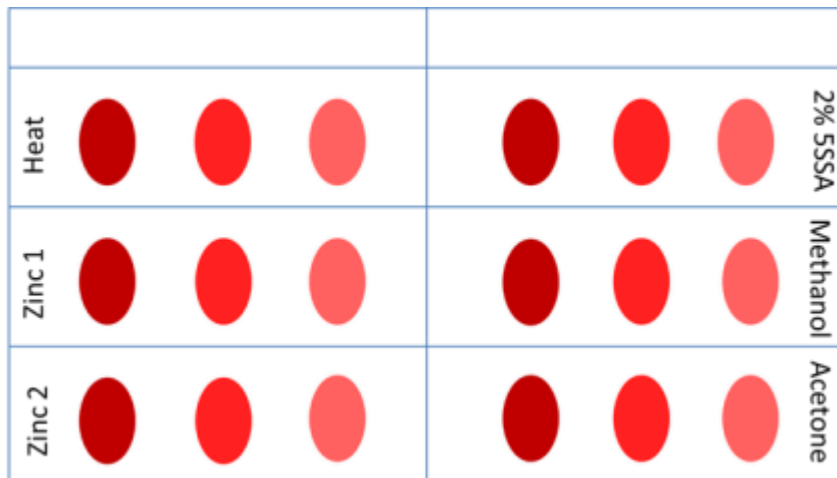


Figure 7. Diagram of the bloody fingerprints applied on the painted metal pieces used in the evaluation of the six fixatives.

2.4 Evaluation of 5-Sulfosalicylic Acid Fixative on Glass and Metal Surfaces

The second part of the experiment acted as a control, in which fingerprints fixed with 5-SSA and non-fixed fingerprints were processed using the same enhancements to show the effect of the fixative. Glass slides were used to simulate glass windows in cars and 4 inch by 6 inch rectangular metal pieces were cut from sheets of painted aluminum metal to simulate the painted metal found on cars.

For the glass portion of the experiment, 16 clean slides were labeled according to the chemical enhancement that would be used. Four trials were conducted for each of the 4 chemical enhancements. Due to the small size of the slides, one trial was conducted per slide. A diminishing series of 3 bloody fingerprints was applied to each of the slides, as shown in Figure 8, using the same procedure as in the first part of the experiment. After the prints had dried overnight, the slides were first fixed using 5-SSA for five minutes using the toweling method, followed by chemical enhancement for three minutes using the toweling method. One of each set had a control slide to which no fixative was applied.



Figure 8. Image of glass slide containing diminishing series of bloody fingerprints.

For the painted metal portion of the experiment, four metal cut-outs were cleaned using a damp paper towel, allowed to dry, and labeled according to the chemical enhancement that

would be used. Multiple trials were performed on each piece of metal using one chemical enhancement method per piece. Six sets of diminishing prints were applied to the metal using refrigerated bovine blood; three sets of prints on the left and three sets of prints on the right. Figure 9 depicts the placement of the six sets of prints on each piece. The prints were allowed to dry overnight before processing. The three sets of prints on the left were enhanced using one of the four chemical reagents using the toweling method for three minutes. No fixative was applied to the prints on the left as a control. The prints on the right were first fixed with 5-SSA for five minutes using the toweling method and then one of the four chemical enhancements was applied for three minutes using the toweling method.

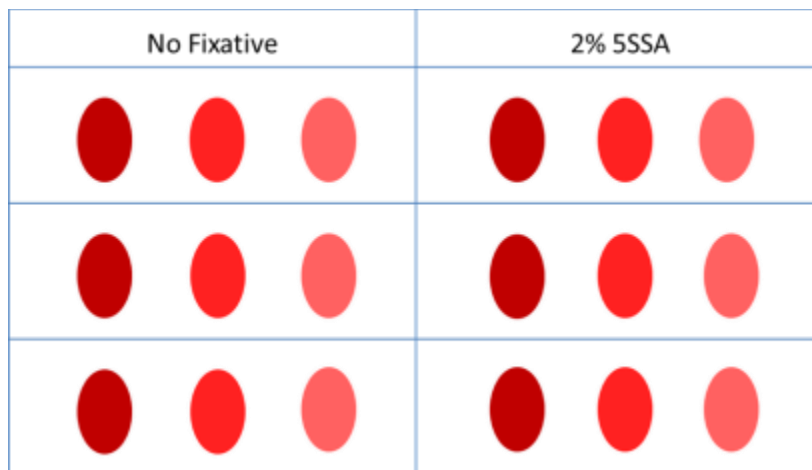


Figure 9. Diagram of the diminishing series of bloody fingerprints applied on the painted metal car pieces.

2.5 Evaluation of Current Processing Methods on Contaminated Surfaces

The purpose of the third part of the experiment was to evaluate the effectiveness of the entire processing method, both fixing and enhancement, of bloody fingerprints on a variety of contaminated surfaces. The enhancement methods used were four common

dyes used in the enhancement of bloody friction ridge impressions: Leucocrystal Violet (LCV), Amido Black, Hungarian Red, and Acid Yellow 7. The LCV solution used in this experiment was prepared in the lab and consisted of 3% hydrogen peroxide, 5-sulfosalicylic acid and sodium acetate.⁽¹⁵⁾ Since the LCV solution contained a fixative, an additional fixation step was not required, but for consistency, all impressions were fixed before processing with any chemical enhancement reagent. For the Amido Black stain, a pre-mixed aqueous solution was used in this experiment containing acetic acid and formic acid.⁽²⁵⁾ The Hungarian red dye in this experiment was a pre-mixed solution containing acetic acid was used.⁽¹⁶⁾ The Acid Yellow 7 solution was prepared in the lab using acetic acid and ethanol. This enhancement reagent required a specific rinse solution which was composed of 5% acetic acid, 25% ethanol, and 70% distilled water.⁽²⁴⁾ The composition and manufacturers of the various chemical enhancements used in this experiment can be found in Table 1.

Table 2. List of chemical enhancement reagents.

Reagents	Contents	Manufacturer
Leucocrystal Violet	100 mL 3% Hydrogen Peroxide 2g 5-Sulfosalicylic Acid 0.74g Sodium Acetate 0.2g Leucocrystal Violet	Aaron Health ACROS Organics FISHER ACROS Organics
Amido Black	Pre-mixed solution	Evident
Hungarian Red	Pre-mixed solution	BVDA
Acid Yellow 7	0.1 g Acid Yellow 7 5 mL Acetic Acid 25 mL Ethanol 70 mL Distilled Water	Arrowhead Forensics ACROS Organics Pharmco-Aaper -

The contaminants used were substances that would commonly be found on the surfaces of vehicles. These included environmental contaminants such as pollen and dust, road contaminants such as salt, mud and motor oil, and applied protection and correction coatings such as car polish and car wax. Glass slides were once again used to simulate glass windows found in vehicles and several painted metal cut outs from cars were used as a sample of the various painted metal surfaces on a vehicle.

For most of the tests, bloody prints were deposited on the surfaces both before and after the application of the contaminants. In some cases, the bloody prints were only applied after the contaminants were on the surface. This was due to the fact that the application of certain contaminants, such as mud, car wax, and car polish, would destroy or wipe away the prints and therefore nothing would be available to process or enhance. The contaminants and bloody fingerprints were allowed to dry completely before the subsequent application of prints or contamination. All of the items were photographed and allowed to air-dry overnight before processing.

For the glass portion of this experiment, separate slides were used for each set of three diminishing prints. For the painted metal portion, six trials were performed on each piece (three with prints under the contaminant and three with prints over the contaminant), with one piece per contaminant and enhancement mixture. Figure 10 depicts a piece of metal that would be subjected to one type of contaminant and one chemical enhancement method.

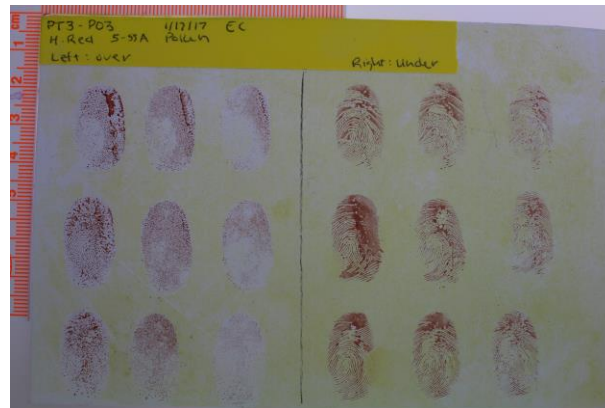


Figure 10. Image of metal piece with contamination applied before and after the deposition of bloody prints.

2.5.1 Environmental Contaminants

2.5.1.1 Pollen

Pine tree pollen was purchased from an online source (Lost Empire Herbs; Soquel, CA). A small amount of pollen was sifted three times through a fine wire mesh sieve into a large weigh boat or container. The glass slides and metal pieces were laid surface side down into the sifted pollen for a few seconds with mild pressure applied. The items were then lifted out of the pollen and tapped on the paper covered lab bench to remove excess pollen, leaving behind a thin layer. The bloody finger prints were deposited both before and after the application of the pollen.

2.5.1.2 Dust

Dust was collected from a household vacuum cleaner and stored in a plastic bin. The dust was dusted or sprinkled onto the surfaces using gloved fingers. Excess dust was tapped off onto a paper covered benchtop. The bloody finger prints were deposited both before after the application of the dust.

2.5.2 Road Contaminants

2.5.2.1 *Salt*

A saturated salt water solution was prepared using unused road salt acquired by the city of Boston for use on city streets and sidewalks. The salt solution was decanted into a small fine mist spray bottle and stored for use. Each slide was spritzed 5-7 times from about 4-6 inches out until a fine layer of water droplets was visible on the surface. When dried, a thin layer of salt crystals was left behind on the surfaces. The bloody prints were applied both before and after the application of the salt water.

2.5.2.2 *Motor Oil*

A few drops of motor oil (Quaker State; Houston, TX) were deposited onto the surface of each substrate using a disposable pipette. The item was tilted to spread the oil over the surface completely. Excess oil was allowed to drip off the item and then the item was gently blotted with a clean paper towel so that only a thin layer of oil remained. Bloody prints were deposited before and after the application of the motor oil.

2.5.2.3 *Mud*

Mud was collected from the side of the road on a rainy day a few blocks from the Boston University Medical Campus and stored in a refrigerator until use. The mud was smeared onto the surface of the substrate and left to dry completely. The bloody fingerprints were deposited only after the mud had been applied to the slide and dried completely. Several attempts were made to apply the mud over the diminishing series of bloody fingerprints,

but each attempt led to the prints being washed away during application of the mud due to its high water content.

2.5.3 Protection and Correction Coatings

2.5.3.1 *Car Polish*

The car polish (Nu Finish; Chicago, IL) was applied to the surfaces of the substrates based on the manufacturer's instructions.⁽²⁶⁾ A small amount of car polish was deposited on a damp paper towel. The paper towel was used to smear the polish onto the surface of each item. The polish was allowed to dry to a white film and then was rubbed off using a low-lint laboratory wipe. No visible residue was left behind. Bloody prints were only deposited after the contaminant had been applied and wiped away, due to the destructive nature of the polish application process.

2.5.3.1 *Car Wax*

The car wax (Turtle Wax; Addison, IL) was applied to the surfaces of the substrates based on the manufacturer's directions.⁽²⁷⁾ A small amount of car wax was deposited onto a damp paper towel which was used to smear the product onto the surfaces of the items. The wax was allowed to dry until a white film was observed and then was wiped away using a low-lint laboratory wipe. A visible waxy residue was left behind after wiping. Bloody fingerprints were only deposited after the contaminant had been applied and wiped away due to the destructive nature of the waxing application process.

2.6 Evaluation of Current Methods Using Winter Road Slush Contaminants

Several slush mixtures were collected from the road from three locations around Boston after snow had fallen. The snow slush was usually collected the morning after the snow storm to ensure that the snow had been driven through and the roads had been salted so that the slush contained a variety of contaminants that can be found on the exterior of cars in winter. All of the slush mixtures were pooled together and poured into a spray bottle for application to the substrates being tested.

Four painted aluminum pieces, the same that were used in previous experiments, were sprayed thoroughly with the slush mixture until the surface was coated with visible droplets. The metal pieces were stored flat laying face-up and left to dry overnight. The next day, six sets of three diminishing bloody fingerprints were applied over the dried contaminant layer, as shown in Figure 11. These prints were left to dry overnight before processing.



Figure 11. Image of the metal piece with bloody prints applied over dried road slush contaminant. All bloody fingerprints applied over contamination.

The bloody fingerprints were first fixed using 5-SSA which was applied using the toweling method for five minutes. After the fixative was rinsed off with tap water one of the four enhancement reagents was applied to the metal piece using the toweling method for three minutes before being rinsed. This process was repeated for each of the four metal pieces using one enhancement method per piece.

2.7 Grading System for Developed Fingerprints

After the enhanced fingerprints were dried and photographed, they were graded using a grading scheme recommended by the Centre for Applied Science and Technology (CAST), shown in Table 3.^(28, 29) This allowed the number and quality of the enhanced fingerprints to be evaluated in a relatively consistent manner.

Table 3. Grading scheme for the assessment of developed fingerprints.

Grade	Level of Detail
0	No evidence of print
1	Some evidence of contact but no ridge detail present
2	Less than 1/3 of print showing clear ridge detail
3	Between 1/3 and 2/3 of print showing clear ridge detail
4	Over 2/3 of print showing clear ridge detail

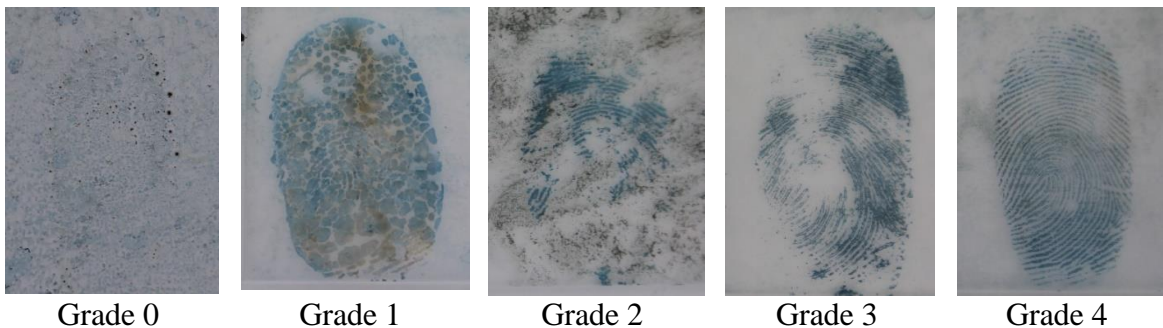


Figure 12. Examples of developed fingerprints corresponding to each grade.

3. RESULTS AND DISCUSSION

3.1 Challenges with Deposition of Bloody Prints on Contaminated Surfaces

When comparing the ease of deposition and adherence of bloody prints applied to the two substrates, the bloody prints tended to deposit more cleanly on the glass slides. The painted aluminum metal was a smoother substrate and when the prints were applied the adhesion to the surface did not overcome the cohesion of the blood to itself as effectively as it did on the glass substrate. Because of this, the blood would slightly pool together which in turn affected the transfer of the ridge detail. Also, the bloody prints adhered very well on the glass slides but would flake off of the painted metal surfaces when dried.

In addition to the type of substrate, there are several other factors that could affect the deposition of a bloody fingerprint and subsequently the outcome of the enhancement of that print. Some of these variables include: temperature, the amount of blood on the finger, the texture and angle of the surface it is being applied to, deposition pressure, and the dryness of the blood on the finger prior to deposition. When there is an abundance of blood on the pad of the finger it is almost impossible to deposit a fingerprint that contains any ridge detail. Just enough blood is needed to coat the finger pad and even then the blood has to be partially dried before an exploitable fingerprint can be deposited. The drier the blood on the finger, the more pressure is needed to transfer the friction ridge detail. If the blood is completely dry, though, no blood will transfer. Temperature and airflow, as well as the amount of blood on the finger, can affect drying times and therefore the deposition of the print. All of these factors combined will ultimately affect

the appearance of the transferred pattern.⁽³⁰⁾ In lab settings it is difficult to control or account for some of these conditions so the prints being studied may vary significantly or be otherwise less than ideal. In the course of a crime, it can be assumed that neither the assailants or victims are concerned with these factors, so it is important to understand that bloody impressions discovered at crime scenes may not be found in the best conditions, irrespective of any contaminants that may come in to contact with the surface before or after the prints have been deposited.

It was difficult to deposit good quality prints each time a new substrate was tested, which is why three trials were performed for each method. The goal was to try to deposit prints that would require enhancement so it was hard to tell sometimes whether the initial print was poor or if the print was not fixed properly and was therefore damaged during processing.

3.2 Evaluation of Various Fixatives on Bloody Impressions

The first part of the experiment was intended to determine whether 5-SSA was an effective fixative or if there was another fixative that worked just as well or better and could be used in later experiments. For the glass portion of the experiment, most of the fixatives resulted in a print that appeared to be fixed, except for acetone. When acetone was applied using the toweling method the prints washed away completely (Figure 13). Since no prints remained on the slide after fixing with acetone, they were not able to be further processed.

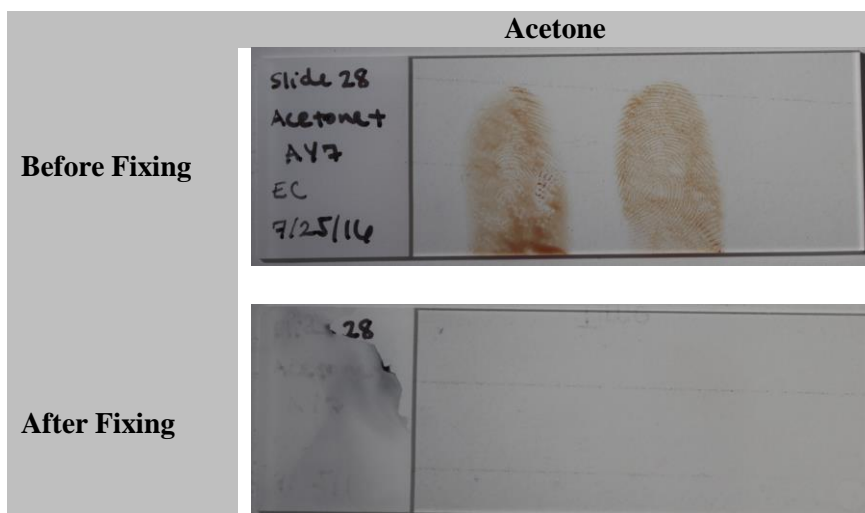


Figure 13. Results using acetone as a fixative for blood on glass slides.

Following enhancement, the only fixative that was observed to work effectively for each of the enhancement methods was 5-SSA. When the zinc fixatives and heat were used, neither the Amido Black nor Acid Yellow 7 was able to stain the bloody prints effectively. In those cases either no ridge detail or no print was observed after processing. Methanol fixing resulted in a very faint Amido Black stained impression and no ridge detail seen in the print stained with AY7. All of the fixed prints were effectively stained with LCV and Hungarian Red, since they did not wash away during the application of chemical enhancement. Since LCV contains 5-SSA in its solution, that may have help to fix the blood while the print was being stained (Figure 14).

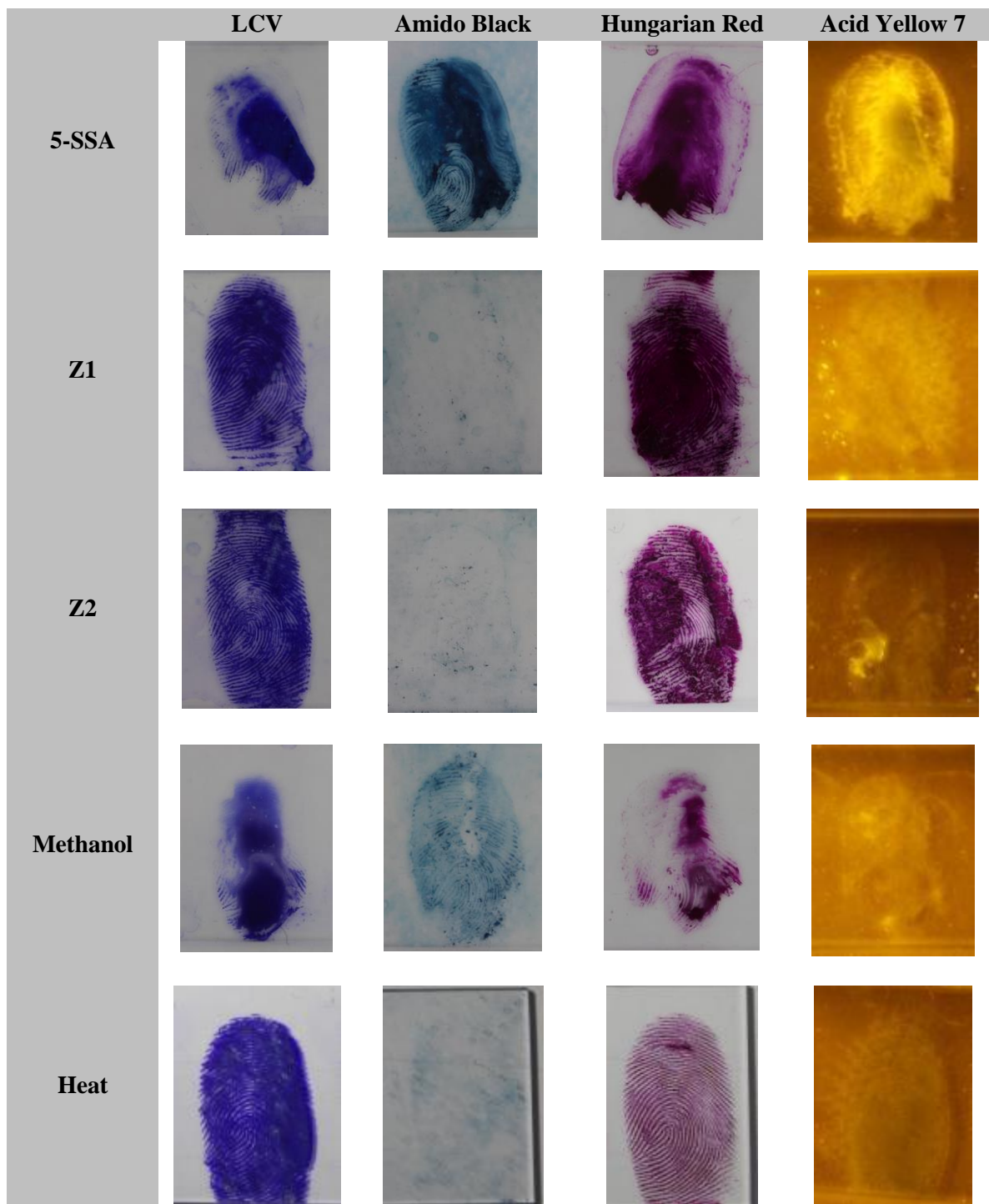


Figure 14. Results of the effectiveness of various solutions in the fixing of bloody impressions made on glass slides.

For the metal portion of this experiment, six sets of bloody prints were applied to each piece of metal tested. Similar to the results observed using the glass slide, the acetone fixative completely washed away all of the ridge detail from the surface after the five-minute fixing time (Figure 15).

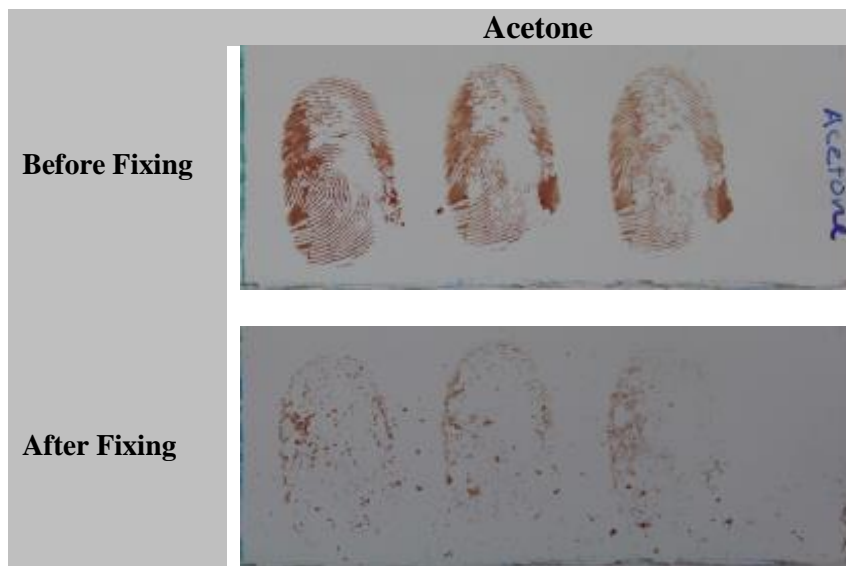


Figure 15. Results using acetone as a fixative on painted aluminum metal.

An interesting observation was made for one of the metal substrates that was not observed in the glass slide portion of the experiment. On one of the painted metal pieces the methanol removed the faintest print in the series (Figure 16). It is not clear why this occurred but it shows that fixing with methanol may, in some instances, be destructive and should be applied with caution.

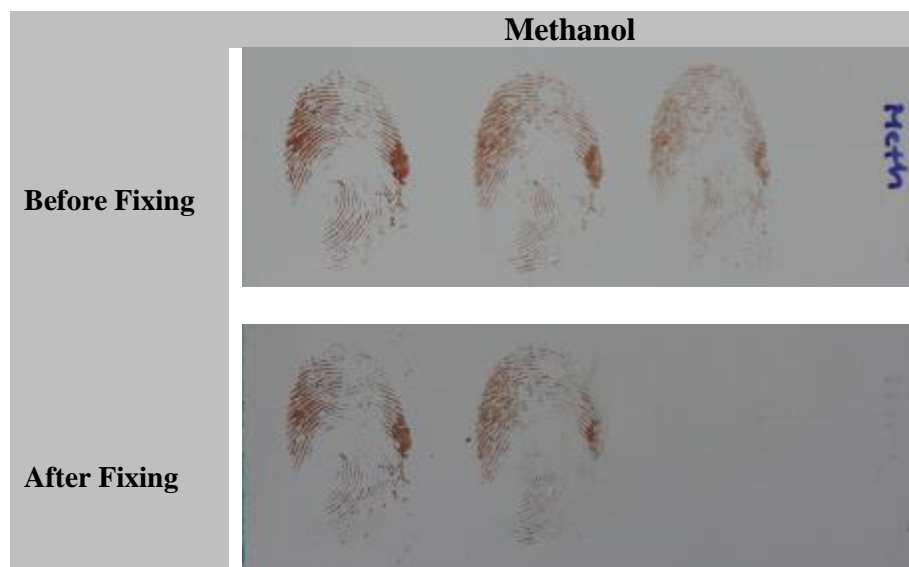


Figure 16. Results using methanol as a fixative on one of the painted metal pieces.

A discoloration effect was observed for the prints that were fixed with 5-SSA, Z1 and Z2. After the five-minute fixing period, the prints that were fixed with those three fixative solutions turned a dull brownish color, while the prints fixed with either the heat or methanol remained a relatively vibrant reddish color. The prints fixed with 5-SSA appeared visually more brown than the ones fixed with either of the zinc solutions (Figure 17).



Figure 17. Discoloration of bloody prints after application of 5-SSA fixative. Left: Before fixing. Right: After fixing with 5SSA.

After the bloody impressions were fixed with the various solutions, chemical enhancements were applied and the effectiveness of the staining was observed. All of the prints, besides the ones that were washed away in the fixing step, contained visible ridge detail upon staining. It was interesting to note that fewer prints were destroyed after processing on painted metal aluminum than on glass substrates. The prints fixed with 5-SSA prior to enhancement exhibited more crisp ridge detail than those fixed with other solutions. In comparing the two zinc solutions, the Z1 solution performed better than the Z2 solution, contrary to a previous study.⁽²⁰⁾ When analyzing the AY7 photographs, the prints fixed with 5-SSA exhibited the most fluorescence and visible ridge detail compared to the others. The fluorescence was muted for all of the prints on metal, however, compared to the AY7 prints on the glass slide.

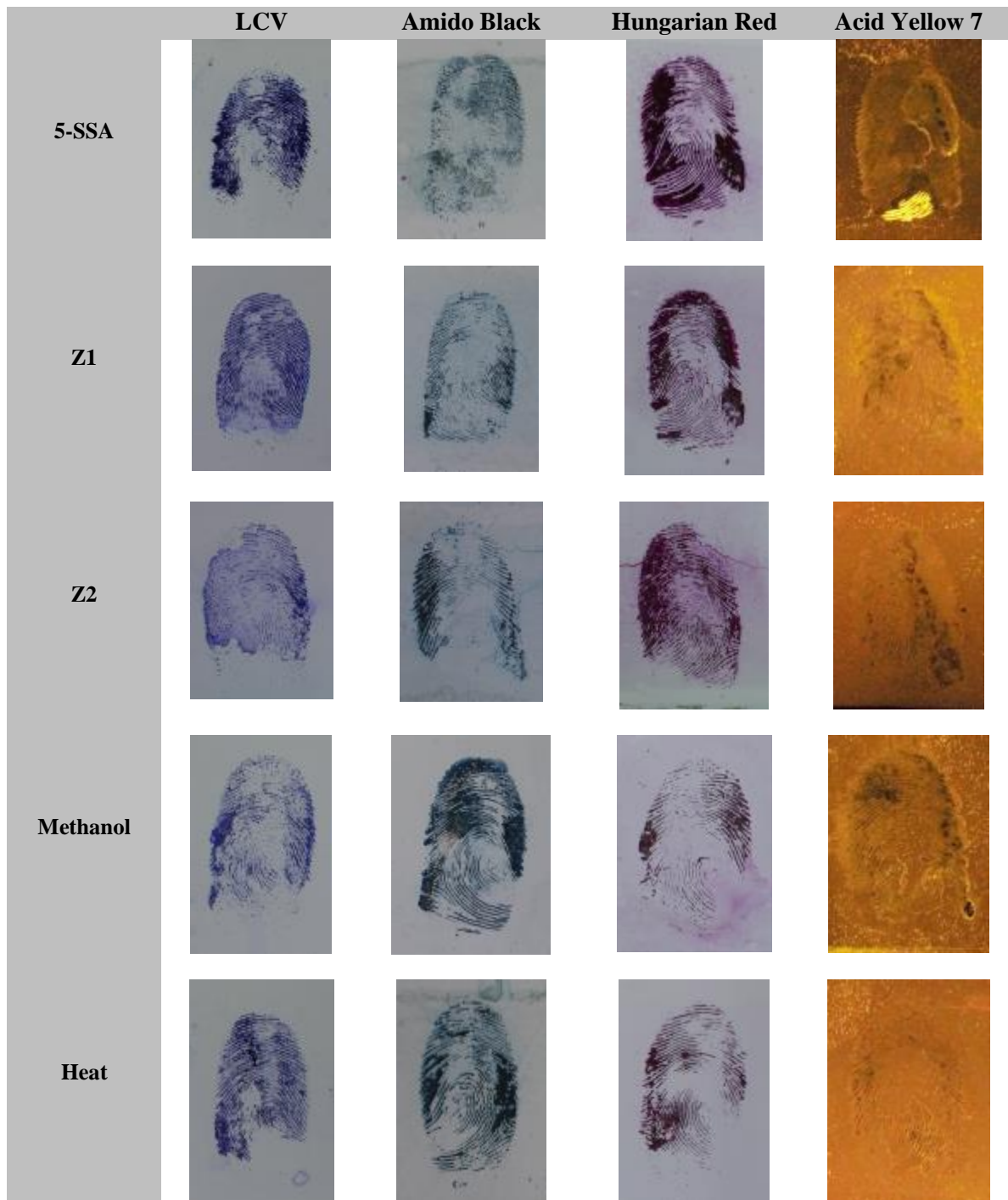


Figure 18. Results of the effectiveness of various solutions in the fixing of bloody impressions made on painted aluminum metal. Photos taken after processing.

3.3 Results of Current Processing Methods with Various Contaminants

3.3.1 Pollen

The processing of the prints applied prior to the application of the pollen was largely unaffected by the presence of the pollen. The application of the bloody fingerprints on top of the pollen proved to be nearly impossible after the first print in the series was placed. The pollen would adhere to the blood on the pad of the finger and would negatively impact the transfer of next print in the series. It appeared that a blood-pollen mixture was transferred to the surface, but the pattern of the ridges was obscured. Because of this, the prints on top of the contaminant would not be sufficient for comparison even after processing.

For the prints applied to both the glass and the painted aluminum metal substrates, the pollen was effectively rinsed off of the substrate after the fixative step and did not seem to hinder the enhancement process. The only issue resulted from the deposition of the prints on top of the pollen which affected the amount of ridge detail that was available after the blood had been stained. In the case of the AY7 stain, more contrast was observed between the background and the fluorescing print on the glass slides than on the painted aluminum. This may suggest that the coating on the metal exhibits its own fluorescence and succeeded in washing out the fluorescence emitted by the stained print.

3.3.2 Dust

The prints applied prior to the dust seemed to be unaffected by the dust during processing. The application of the prints on top of the dust proved to be more difficult, especially by the third print in the series since the dust would cling to the blood on the finger pad and prevent the transfer of some of the blood for the next print in the series. After processing, the first two prints in the series seemed to have mostly transferred and been processed effectively. The third print in the series processed poorly, mainly due to the lack of transfer of the blood to the item.

3.3.2.1: Glass Slides

The rinse after the fixative step worked to effectively rinse most of the dust off of the surface so it did not interfere with later processing. For the most part, the prints applied on top of the dust resulted in stained prints that were vibrantly dyed and exhibited crisp ridge detail. The prints deposited before the dust was applied appeared fainter in color after processing, except for the AY7 dyed prints. These prints exhibited much more fluorescence than those applied on top of the contaminant. This may suggest that, while the dust was effectively rinsed off of the glass slide it may have adhered to the bloody print. This may have contributed to the faintness of the prints dyed with LCV, AB, and HR. Since dust may contain some proteins from various sources and the protein dyes are not blood specific, the additional fluorescence in the AY7 may have been due to the dust adhering to the ridge pattern.

3.3.2.2: Painted Metal Pieces

After the fixative was rinsed off, dust still adhered to the surface of the metal substrate. The processing using LCV was unaffected by this, but all of the protein dyes stained the dust remaining on the surface providing poor contrast between the enhanced prints and the background. The prints deposited before the application of dust fared better than the ones applied on top of the dust. The visibility of prints enhanced with AY7 was hindered by the fluorescence of the dust remaining on the surface of the metal and the ridge detail was almost completely washed out.

3.3.3 Salt

Salt seemed to have a variety of deleterious effects on bloody fingerprints. The prints deposited over the salt would exhibit missing ridge detail after processing. It appeared that once the salt crystal washed away after the various rinses and stains there would be a hole in the print in that location. Since that portion of the print was on the salt rather than the slide, the blood would wash away with the salt. The ridge detail would appear smudged after being applied over a layer of salt and being left to dry overnight. It is not clear if this was due to some reaction between the salt and the blood or if the salt crystals merely affected the deposition of the print.

The level of detail and crispness of the processed prints deposited before the salt water was applied seem to depend on the spray of the solution. The ridge details tended to smudge if the spray wasn't fine or if it was applied too often or too close to the surface of

the substrate. Since blood is water soluble a splash up of salt water from the road could potentially wash away prints.

3.3.4 Motor Oil

3.3.4.1: Glass Slides

Prints applied on top of the oil held up very well on the glass substrates. The impression of ridges into the soft oil seemed to hold the pattern of the bloody print in place. This resulted in crisp ridge detail and almost perfect impressions after processing with all four enhancement reagents.

The prints deposited before the motor oil was applied did not process as well. The processed prints appeared spotty and sections of the pattern of the ridge detail seemed to disappear in various places across the print.

3.3.4.2: Painted Metal Pieces

For the painted aluminum substrate, bloody fingerprints were nearly impossible to deposit on top of the motor oil. When an attempt was made to deposit a print, the blood pooled into small droplets on top of the oil. This is due to the fact that, like water and oil, blood and oil do not mix due to their opposing polarities. When these prints were processed only the bubbles of blood stained and no ridge detail was seen. It is unclear why the prints applied on top of the motor oil on the glass substrate retained the ridge detail made in blood while those applied on the painted aluminum substrate did not.

The prints deposited before the application of the motor oil exhibited the same effects as the ones similarly applied to the glass slides. The prints appeared spotty and ridge detail when missing in various locations. In addition, it appeared that the bloody ridges may have been lifted off the substrate in the oil but due to their inability to mix, the suspended ridges were shifted in pieces for short distances. This can be seen in the Hungarian red photo in Table 11.

3.3.5 Mud

Bloody prints were very difficult to apply on top of dried mud for both the glass and painted aluminum substrates. The mud did not easily wash off after any of the rinsing or staining steps and only the portions of ridge detail that came into contact with the substrate were enhanced. Any blood that was deposited on top of the mud was washed away. In addition, all of the dyes, except LCV, heavily stained the mud remaining on the surface leading to poor contrast of the available ridge detail. The portions of the fingerprints that made direct contact with the substrate and were stained appeared crisp, however.

3.3.6 Car Polish

After the car polish was applied and removed the surfaces of the substrates appeared to be shinier even though no residue was observed to be left behind. Besides the more difficult deposition of the bloody prints, the contaminant appeared to have no effect on the processing of bloody prints on glass surfaces. In the case of the painted aluminum

substrate, the deposition of the print on the slippery surface proved to be more difficult and the blood did not adhere to the surface in the transferred pattern as well. This resulted in faintly stained prints for LCV, AB, and HR. No ridge detail was observed in those stained with AY7 since intense the background fluorescence washed out the faint fluorescence of the print.

3.3.7 Car Wax

Since a waxy residue was left behind after the contaminant was applied, the application of the prints was not as clean. The blood would not adhere to the slippery surface as well as a clean dry surface and some pooling was observed. For the most part the patterns held. On the glass surfaces, the processed prints appeared crisp and vibrantly stained. On the painted aluminum surfaces, the stained prints were faint, but ridge detail was visible after processing with each of the four chemical enhancements.

3.4 Results of Current Processing Methods with Winter Road Slush Contamination

The slush mixture collected from the road appeared to contain salt, dirt, oil and a variety of other unknown contaminants. The application of the bloody fingerprints on top of the dried slush mixture was similar to the deposition of prints on top of the substrates contaminated with the salt solution. Salt crystals adhered to the metal and only slightly affected the transfer of the ridge pattern. The contamination did not appear to have an effect on the processing of the bloody prints. The processed prints retained the ridge detail observed before processing and appeared to have crisp ridges.

3.5 Results of Enhanced Fingerprints Using the Grading Scheme

For each set of prints, a diminishing series of three bloody fingerprints was deposited onto various contaminated surfaces. Each print in the set was evaluated for its quality based on the grading scheme described in Table 3. Since a total of three trials were performed for each experiment, the grades in the Tables represent an average of the same print in the series for the three trials. Tables 4-5 and 6-7 show the grades for the fingerprints developed on glass slides and metal pieces, respectively.

Table 4. Results of enhanced prints applied under contaminant on glass slides. Average score for each print in the diminishing series (1 is the first print deposited, etc). Each series was performed in triplicate. Controls were not exposed to contaminants.

Print #	LCV			Amido Black			Hungarian Red			Acid Yellow 7			Avg
	1	2	3	1	2	3	1	2	3	1	2	3	
Pollen	4.0	2.3	2.0	3.0	4.0	4.0	2.3	3.3	4.0	3.0	2.3	3.0	3.2
Dust	3.7	3.0	2.7	4.0	3.0	2.7	2.7	3.0	2.7	2.0	2.3	3.0	2.9
Salt	3.7	2.7	3.3	1.3	2.0	2.3	2.0	2.3	2.7	3.3	3.0	3.3	2.7
Motor Oil	2.3	2.3	3.0	1.0	1.7	3.0	2.0	2.3	3.3	1.0	1.7	1.7	2.1
Average	2.9			2.8			2.7			2.5			
Control	2.0	2.7	4.0	3.0	3.0	3.7	2.3	2.7	3.7	2.3	2.3	3.3	2.9
Cont. Avg	2.9			3.2			2.9			2.6			

For bloody fingerprints applied underneath a contaminant on glass, the processing methods were least affected when the prints were applied under pollen and dust. Salt and motor oil appeared to have somewhat of a detrimental effect on subsequent processing compared to the other contaminants. Amido Black and Acid Yellow 7 performed the worst on prints that had been applied prior to contamination with motor oil. Overall, there

was a negligible difference between the average grades of the four chemical enhancement methods used on bloody prints applied underneath these particular contaminants (Table 4).

Table 5. Results of enhanced prints applied over contaminant on glass slides. Average score for each print in the diminishing series (1 is the first print deposited, etc). Each series was performed in triplicate. Controls were not exposed to contaminants.

Print #	LCV			Amido Black			Hungarian Red			Acid Yellow 7			Avg
	1	2	3	1	2	3	1	2	3	1	2	3	
Pollen	3.0	2.3	1.7	2.3	2.7	2.7	2.0	2.7	2.7	3.3	2.7	1.7	2.5
Dust	3.7	3.0	3.0	4.0	3.7	3.0	3.3	3.3	3.3	3.0	3.0	3.7	3.3
Salt	2.7	2.3	2.3	2.3	2.0	2.7	2.7	2.3	2.7	3.7	2.7	2.3	2.6
Motor Oil	4.0	4.0	3.7	4.0	4.0	3.7	2.3	2.7	2.3	4.0	4.0	4.0	3.6
Mud	2.7	1.7	1.0	2.7	2.0	1.7	2.3	1.7	1.3	1.7	0.3	0.0	1.6
Car Wax	2.7	3.3	3.3	3.3	3.3	3.7	3.7	3.3	3.3	3.3	2.7	2.3	3.2
Car Polish	3.7	3.3	2.7	3.7	3.3	3.0	3.3	3.3	3.3	4.0	3.7	3.0	3.4
Average	2.9			3.0			2.8			2.3			
Control	2.0	2.7	4.0	3.0	3.0	3.7	2.3	2.7	3.7	2.3	2.3	3.3	2.9
Cont. Avg	2.9			3.2			2.9			2.6			

The bloody fingerprints on glass that were applied over the dried mud proved the hardest to process regardless of which chemical enhancement was used. The fingerprints that were processed with Acid Yellow 7 on the muddy surface were rarely able to be visualized, though, while the processing with the other three reagents resulted in some visible ridge detail. Overall, there seemed to be a negligible difference between the average grades of three of the four chemical enhancement methods -- LCV, Amido Black

and Hungarian Red -- used on bloody prints applied on top of the contaminated surfaces in this experiment (Table 5).

Table 6. Results of enhanced prints applied under contaminant on metal pieces. Average score for each print in the diminishing series (1 is the first print deposited, etc). Each series was performed in triplicate. Controls were not exposed to contaminants.

Print #	LCV			Amido Black			Hungarian Red			Acid Yellow 7			Avg
	1	2	3	1	2	3	1	2	3	1	2	3	
Pollen	2.7	2.7	2.7	3.7	3.3	2.7	3.7	3.3	3.0	2.3	2.3	2.0	2.9
Dust	2.7	3.7	4.0	2.7	3.7	3.3	2.0	3.0	3.3	0.0	0.0	1.0	2.5
Salt	2.7	2.7	2.3	2.7	3.0	2.7	3.7	2.7	2.7	1.3	2.0	2.0	2.5
Motor Oil	2.0	2.3	2.0	2.0	2.0	1.7	2.0	2.0	1.7	1.7	1.3	1.3	1.8
Average	2.7			2.8			2.8			1.4			
Control	3.3	3.0	3.3	3.0	3.0	2.7	3.3	3.0	2.7	3.0	2.0	2.7	2.9
Cont. Avg	3.2			2.9			3.0			2.6			

For bloody fingerprints applied under contaminants on metal, the Acid Yellow 7 reagent performed the worst out of all four reagents tested, resulting in little to no ridge detail in the bloody impressions that were applied prior to the dust, salt and motor oil contaminants. Pollen had less of an effect on the subsequent processing of bloody fingerprints using Acid Yellow 7. All four of the chemical enhancement methods tested were less effective when a motor oil contaminant was applied over the bloody impressions, usually resulting in less than 1/3 of the print showing clear ridge detail after processing. The average grades showed no significant difference for LCV, Amido Black, and Hungarian Red (Table 6).

Table 7. Results of enhanced prints applied over contaminant on metal pieces. Average score for each print in the diminishing series (1 is the first print deposited, etc). Each series was performed in triplicate. Controls were not exposed to contaminants.

Print #	LCV			Amido Black			Hungarian Red			Acid Yellow 7			Avg
	1	2	3	1	2	3	1	2	3	1	2	3	
Pollen	1.7	1.7	1.7	1.3	1.3	1.7	2.0	1.7	2.7	1.0	1.0	1.0	1.6
Dust	3.7	2.7	2.3	3.7	2.3	1.7	3.7	2.0	1.7	0.0	0.0	0.0	2.0
Salt	3.7	2.3	2.0	3.0	1.7	3.0	3.3	2.3	2.0	1.3	1.0	1.0	2.2
Motor Oil	1.0	1.0	1.0	0.0	0.0	0.3	1.0	0.0	0.3	0.0	0.0	0.0	0.4
Mud	2.3	0.7	0.3	2.0	0.7	0.0	2.3	0.7	0.0	0.7	0.7	0.0	0.9
Car Wax	2.7	2.3	2.3	2.7	2.7	2.3	2.7	2.0	2.7	1.0	1.3	1.7	2.2
Car Polish	2.7	2.3	2.0	1.7	2.0	2.3	2.0	2.0	2.0	0.0	0.0	0.0	1.6
Road Slush	3.0	2.7	2.3	3.3	2.7	2.0	3.0	2.7	2.0	2.3	2.7	2.0	2.6
Average	2.1			1.9			2.0			0.8			
Control	3.3	3.0	3.3	3.0	3.0	2.7	3.3	3.0	2.7	3.0	2.0	2.7	2.9
Cont. Avg	3.2			2.9			3.0			2.6			

All of the enhancement methods performed poorly on bloody fingerprints that had been applied on metal on top of the motor oil contaminant. This was mainly due to the inability to deposit a fingerprint on the oil that was coating the painted metal surface. Mud also affected the processing of the bloody fingerprints with all four chemical enhancements. This may have been due to the inability of the bloody impression to make contact with the metal surface through the layer of mud. Since it could not make contact, it could not be fixed to the painted metal surface and was washed away. The Acid Yellow 7 reagent performed the worst out of all four reagents tested, resulting in little to no ridge detail in processed fingerprints. The average grades showed no significant difference for the other three chemical enhancements that were used to process the bloody impressions.

4. CONCLUSIONS

The 2% 5-Sulfosalicylic acid fixative solution performed the best out of all of the fixatives tested in this experiment. With the exception of subsequent processing with LCV and Hungarian Red, the other fixatives did not appear to effectively fix the bloody impressions to the surface. The LCV solution itself contains 5-SSA, which may have led to the impression adhering to the surface regardless of the applied fixative used. It is unclear if the Hungarian Red solution contains any fixatives due to the proprietary nature of the composition of the pre-mixed solution.

The combination of 5-SSA fixative with the LCV enhancement reagent performed the best overall out of all four enhancement methods, especially in the experiments involving contaminated surfaces. Due to its initial colorless state and its specificity for hemoglobin rather than other proteins, it never reacted with any of the contaminants or stained the background of either substrate. Hungarian Red was also a suitable enhancement method in that even when the background was stained, the contrast between the print and the substrate was still acceptable. In addition, bloody prints stained with Hungarian red have the ability to be lifted on to a white gel lift and observed using an ALS to further improve contrast.⁽¹⁶⁾

The contrast of the water-based Amido Black dyed prints was poor compared to the other dyes tested in this study since it tended to stain the prints a light grayish-blue color and it produced less crisp ridge detail than both Hungarian Red and LCV after processing.

Similar results were obtained in a previous study comparing various enhancement methods, in which the authors concluded that while the water-based formulation of Amido Black was easy to use and bulk solutions could be stored for long periods of time, it produced very poor results, both on porous and non-porous surfaces, with usable print recovery rates of only 11%.⁽³¹⁾ In addition, the aqueous Amido Black solution tended to react with many of the contaminants applied to the metal and glass surfaces in this experiment and would not be effectively rinsed off after over one minute of rinsing.

When prints treated with AY7 are enhanced effectively the fluorescence has the ability to provide great ridge detail in photographs, and generally works best on faint prints as heavier deposits of blood tend to absorb some of that fluorescence and require longer soaking times.⁽³²⁾ The downside to using a fluorescing dye, however, is that should the substrate exhibit its own fluorescence, the details of the print would be masked or washed out by the competing fluorescence. Some of the contaminants studied, such as the car polish, pollen and dust, exhibited varying degrees of fluorescence on their own and decreased the contrast between the print and the background.

For the painted aluminum metal bloody fingerprints exposed to 'winter road slush', the conclusions were similar to that of a previous study in which the authors examined the recovery of latent prints on surfaces exposed to snow. In all cases, some ridge detail was able to be developed.⁽³³⁾ The enhancement of the fingerprints in this study appeared, for

the most part, to be unaffected by the road slush contaminant on the surface of the metal substrates.

Overall, the results suggest that 2% 5-SSA in conjunction with either LCV, Amido Black, or Hungarian Red, are effective for the enhancement of bloody friction ridge patterns applied to the surfaces of vehicles. Compared to the bloody fingerprints in the uncontaminated control samples, the fingerprints processed on the contaminated surfaces showed similar level of detail assessment scores. In some instances the prints on the contaminated surfaces resulted in better scores than the control prints. It was observed that the contaminants mainly affected the deposition of the prints onto various surfaces rather than the subsequent enhancement of the prints. Surface contact plays a role in the ability of the print to be fixed and stained. The print cannot be effectively fixed to a surface if a contaminant is between the fixative and the print. It was shown that a bloody fingerprint could effectively be fixed and stained using the aforementioned methods, given that the transferred pattern had sufficient contact with the substrate on which it was applied and that the pattern initially transferred was of good quality. These enhancements methods provide additional evidentiary value to the photographs taken before enhancements are applied by staining faint impressions that may not be visible to the naked eye.

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