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Assessing Methods to Enhance and Preserve Proteinaceous Impressions from the Skin of Decedents during the Early Stages of Decomposition while Examining Environmental Variations across Seasons

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Abstract

Homicides and violent crimes often result in bloodshed; the constant substrate involved in physical altercations in the commission of violent crimes is human skin. Thus, it is likely blood impressions are left on the skin of living victims or decedents during these violent interactions. Yet, skin is one of the least studied substrates in the impression discipline. In some cases, impressions are clearly visible but, it is much more likely that they are latent and not readily visible. There have been cases where the enhancement of blood impression evidence on human skin was possible, but it is not standard practice, especially when blood impressions are latent. While visible blood impressions are best enhanced in situ at the scene of the crime, most often these impressions on decedents are not enhanced until the body is moved to the medical facility for autopsy increasing the possibility of damage to the impression evidence from handling and/or moving the body. Because it is semi-porous, skin is a difficult substrate to enhance through chemical enhancement methods due to background staining, which may result in suboptimal visualization of the impressions. In addition to the staining of skin, visualization of impression details may be obstructed by competing background patterns, such as dermal scales, hairs, wrinkles, and variations in skin tones. Two commonly used dye stains, Amido Black and Hungarian Red have been used to enhance blood impressions on human skin and a newer method, Zar-Pro™ Fluorescent Blood Lifters have also been used in preliminary studies to effectively lift and enhance blood impressions from decedent skin. A comparative analysis between methods conducted in collaboration with the Forensic Research Outdoor Station at Northern Michigan University assessed the effectiveness of enhancing semen smears and blood impressions on decedent skin during the early stages of decomposition. All three enhancement methods demonstrated effectiveness in recovering proteinaceous materials with Amido Black and Hungarian Red primarily effective as insitu dye stains. The dye-stained impressions were not reliably lifted using BVDA Gellifters® thus not removing the substrate variables that can impede visualization of impressions on skin. The Zar-Pro™ Fluorescent Lifters were able to effectively lift and fluorogenically enhance proteinaceous materials in the form of blood impressions and semen smears from decedent skin through ten days of active decomposition. A statistical assessment of the enhancement methods was conducted amongst examiners to verify the efficacy of results. During the early stages of decomposition, donor skin will deteriorate, thus recoverable impressions will also be degraded and or damaged, yet this degradation is not perilous for the recovery of proteinaceous materials as long as the epidermal skin is still intact. Thus, even during active decomposition, skin, arguably one of the most difficult substrates for impression recovery, can produce viable impressions and the recoverability of this vital evidence can now be re-evaluated by practitioners in the field.

Executive Summary

The goal of this applied research project was to expand the understanding of aged, degraded limited, damaged, or otherwise compromised physical evidence, specifically the enhancement and recovery of semen smears and blood impressions from decedent skin during the early stages of decomposition. A comparative analysis was conducted between the Zar-Pro™ Fluorescent Lifters, and two known dye stains: Amido Black and Hungarian Red. Zar-Pro™ Fluorescent Lifters are a fairly novel enhancement method that have been used to effectively lift, enhance, and preserve blood and semen impressions on a variety of substrates to include human skin from both living and deceased subjects. Amido Black and Hungarian Red are commonly utilized chemical enhancement methods for research on skin and Amido Black has been used on decedent skin in case work. These methods were selected based on past-use and effectiveness, along with affordability and ease and safety of use at crime scenes. The original plan for the research trials was to conduct human decedent studies in both the winter and summer seasons during the semester breaks to evaluate the effect of seasonal variation, i.e. the warmest and coldest months on the recovery of impressions. Due to unforeseen limitations as a result of the COVID pandemic, the research plans were adjusted which only allowed for one seasonal human decedent study which occurred in the summer of 2021 at the Forensic Research Outdoor Station (FROST) on the campus of Northern Michigan University. To adjust for the alteration to the research timeline two additional studies comparing preserved and fresh fetal pig studies were conducted at the Madonna University Forensic Science Research Facility (MUFSRF) on the campus of Madonna University in the fall of 2020 and 2021. The addition of the fetal pig studies allowed for a comparative assessment of the selected enhancement methods between the more easily acquired fetal pig skin as opposed to actual human decedent skin which is only accessible via human donors.

To test the effectiveness of enhancement methods, optimal quality thumbprint impressions were deposited on the skin of fetal pigs and human decedents, along with semen smears in the human decedent trials. The optimization of deposited impressions helps ensure reproducibility and consistency amongst impressions for research purposes, creating baseline impression quality to allow for a comparative assessment of the effect of decomposition on the recovery of blood impressions from decedent skin. As semen is latent by nature the optimization of semen impressions on human skin was more difficult, thus semen smears were utilized in the research trials instead of semen impressions. For the human decedent trials, blood impressions were deposited on the skin of two decedent donors (decedents 1 and 2) in pre-designated areas: neck area (24), arms (24 on left arm and 24 on right arm), and legs (24 on left leg and 24 on right leg), with additional blood impressions deposited on the upper chest (10). In addition to the blood impressions, semen smears were deposited onto the inner thighs (15), totaling 145 impressions per decedent donor with non-impression controls (10) on the forehead. A subset of blood impressions (33) were deposited onto the skin of a third decedent donor, but due to antemortem injuries sustained by this decedent, the decedents body was contorted thus there was not adequate surface area for the deposition of additional impressions. In addition to the lack of surface area, the decedent started to decompose prior to the start of the research trials. For these reasons, decedent donor 3 was deemed incomparable to decedents 1 and 2. Fetal pig trials were also conducted with blood impressions (10 impressions per pig) deposited on to the skin of on six preserved and six fresh pigs, totaling 120 with non-impression controls on the forehead. The non-impression controls were collected from the forehead of the human decedents and fetal pigs using Zar-Pro™ Fluorescent Lifters and BVDA Gellifters® to assess the effect of any inherent fluorescent properties associated with decomposition on the visualization of the enhanced blood impressions and semen smears.

The protein dye stains: Hungarian Red (EA) and Amido Black (EB), and Zar-Pro™ Fluorescent Lifters (EC) were selected for use in these research trials due to enhancement effectiveness, affordability, and the fact that they are easy and safe for crime scene use. Non-toxic, water-based solutions for Amido Black (TriTech Forensics) and Hungarian Red (TriTech Forensics) were utilized in the trials. The dye stains contained fixatives, so no additional fixatives were required for enhancement. A plastic enhancement tray was designed for the application of dye stains for this project to prevent staining outside of the impression area. Once the enhancement tray was applied to the decedent skin, a Student Researcher applied the dye stains directly to the impression area using disposable pipettes. After the blood impression or semen smear were stained, a de-staining solution was applied to the area to rinse away excess stain from the area. For Amido Black 2mL of stain was applied to the impression area for 1 min before 2mL of de-stain solution was used to rinse the impression with an immediate application of white BVDA Gellifters®. Hungarian Red (EB) utilized 1.5mL of stain onto the impression area for 1 min before 1.5mL of de-stain solution was used to rinse the impression with a two-minute post-enhancement interval before the application of black BVDA Gellifters®. Lifting the stained impressions provides another possibility to visualize the enhanced impression by removing subtracted variabilities associated with human skin. Additionally, lifting the stained impressions allows for the collection of physical evidence for transportation back to the laboratory that is not possible with in situ staining alone. Unlike the dye stains, Zar-Pro™ Lifters (TriTech Forensics) do not require a fixative. Prior to use, the Lifters are activated with Zar-Pro™ Activator (TriTech Forensics) and then applied directly to the impression, lifting the impression onto the white lifter for visualization and preservation. The commercially available products purchased for this study will be used according to manufacturer's instructions.

The blood impressions and semen stains were strategically placed onto the skin substrate and aged in situ on pre-determined locations on the body to be enhanced using each of the three enhancement methods at daily collection intervals over the course of ten-days for the human decedent trials and nine-days for both fetal pig trials. In the human decedent trials, the one-hour blood quality control impressions, one-hour semen control smears as well as one-hour non-impression controls were collected at the Forensic Anthropological Research Laboratory (FARL) prior to transportation of the decedents to the Forensic Research Outdoor Station (FROST) facility for field placement. For the fetal pig trials the blood controls were collected at the Madonna University Forensic Science Research Facility (MUFSRF) prior to transportation to their field placement. The remaining blood impressions, semen smears, and non-impression controls were collected in the field at the daily collection intervals. The decedents bodies were photographed prior to and post collection interval during the trials and the blood impressions, semen smears, and non-impression control areas were photographed at each collection interval insitu prior to enhancement and insitu after dye-staining. An hour after recovery the Amido Black dye-stained impressions affixed the white Gellifters were photographed under normal lighting, Hungarian Red dye-stained impression affixed to black Gellifters were photographed under normal lighting and alternate lighting and the proteinaceous impressions affixed to the Zar-Pro™ Fluorescent Lifters were photographed under normal and alternate lighting. Photography of the recovered impressions on the Gellifters and Zar-Pro™ one hour after enhancement were conducted at the FARL for the human decedent trials and at the MUFSRF for the pig trials with a secondary set of photographs taken of the recovered impressions one month later at the MUFSRF.

The digital images from the human decedent trials were labeled and organized into data sets that were sent electronically to two forensic science students trained in latent print analysis and two practicing latent print analysts working on casework in ASCLD (American Society of Crime Lab

Directors) accredited forensic science laboratories. Semen smears recovered from the decedent skin during the human decedent trials were sent electronically to a forensic science students trained in latent print analysis to rate impression detail using only two-point scale (0 meaning no visible proteinaceous materials and 1 meaning visible proteinaceous materials) as well as fluorescent intensity using provided scale standards. Digital images from the fetal pig trials were also compiled into data sets and sent electronically to two forensic science students trained in latent print analysis. The digital images were rated for the impression details (four-point scale with 4 being optimal quality) under normal and alternate lighting, and fluorescent intensity (six-point scale, with 6 being the brightest) under alternate lighting using provided scale standards. Ratings of impression quality and fluorescent intensity are subjective, even with a provided scale so to reduce any indirect subjectivity of results Cohen's Kappa statistical analysis of inter- and intra-examiner reliability was assessed to verify the significance of the results obtained.

Blood impressions or at least proteinaceous fingermarks were able to be visualized on the decedent skin through most of the collection intervals even through the later stages of early decomposition (7-10 days) if the epidermal skin was still present. The use of the dye-stains Amido Black and Hungarian Red to enhance blood impressions insitu on decedent skin allowed for improved visualization of impression details according to the rating assessments. Thus, the impression quality increased from the rating of the impressions prior to and post enhancement. Amido Black was effective as an insitu dye stain through the six to seven-day collection intervals, whereas Hungarian Red was effective as an insitu dye stain primarily through the five-day collection interval, depending on the level of decomposition in the body area. The use of the white and black Gellifters to lift the dye-stained impressions from the decedent skin were not effective as most of the impression details were lost in transfer with the rating assessments decreasing in impression quality from the insitu stained impressions. The Hungarian Red dye-stained proteinaceous materials on the black Gellifters were fluorescent when visualized under alternate lighting ranging in fluorescent intensity from a high of 3.5 when observed one hour after recovery to a high of 5.17 out of 6 on the scale standard one month later. Although the increase in fluoresce was observed, likely due to the reduction of moisture within the stain affixed to the Gellifters which can quench fluorescence, the marked increase in fluorescence intensity dd not improve the visualization of impression details. The Zar-Pro™ Fluorescent Lifters were effective in lifting and enhancing blood impressions from decedent skin through the ten-day collection interval, in part due to their fluorogenic properties. Lifted blood impressions were readily visible on the white background of the Lifters through the three and four-day collection intervals, at which time the impression details were becoming faint and not readily visible under normal lighting. However due to their fluorescent properties' impression details were visible on the Zar-Pro™ Lifters through eight-days with a double lift collected at the ten-day collection interval. The fluorescent properties of the Lifters are optimized once free of moisture thus the fluorescent intensity at one hour range had a high of 4.92 which increased to a high of 6, the highest rating on the scale standard one month later. Unlike Hungarian Red, the fluorescent properties of the Zar-Pro™ lifted impressions translated to higher impression detail assessments when visualized under alternate lighting. The ability to lift the blood impression from the skin substrate also allowed for improved visualization of impression details, which was a limiting factor when using the insitu dye-stains. The limiting factor for the effectiveness of the Lifters was the presence of the epidermal skin, if the epidermal skin was intact the impression could be recovered, albeit biofluid contamination did hinder the visualization of some impression details. During the advanced stages of early decomposition when

only the greasy epidermal skin remained, the Lifters recovered too much biofluid contamination that even though the lifts were brightly fluorescent impression details could no longer be visualized.

All three enhancement methods allowed for the improved visibility of blood impressions on decedent skin with overall impression quality decreasing through the ten-day trial period. This was expected as the breakdown of the skin and artifacts associated with decomposition would naturally affect the recoverability of blood impression from the skin of human decedents during the early stages of decomposition. Cohens Kappa statistical assessment for examiner ratings determined almost perfect agreement for impression detail ratings under both normal and alternate light. This level of agreement was seen in the inter-examiner assessments of student to student, analyst to analyst, analysts to students, as well as in the intra-examiner assessments. Concluding the examiners had significant agreement in their rating of impression details through the human decedent trials. This level of agreement was not seen in the inter-examiner assessment of fluorescent intensity with ranged from moderate to almost perfect agreement amongst the analyst but only moderate to substantial agreement amongst the students. In comparing the analysts to the students, the level of agreement on fluorescent intensity ranged from poor to almost perfect. This level of disagreement was also seen in the intra-examiner assessments ranging from slight to almost perfect depending on the individual examiner ratings. The variation in ratings for fluorescent intensity concludes examiners were not in agreement with fluorescent intensity using the provided scale standard. However, it was interesting to note, the analysts and students were most in agreement amongst each other (analyst to analyst and student to student) regarding the fluorescent intensity ratings. Due to the variation, when providing fluorescent intensity in the trials, a range of fluorescent intensity was reported which reflects the ratings of all examiners.

The semen smears produced similar results to the blood impression trials on human decedent skin with all the smears effectively enhanced through the five-day collection interval. The student examiner rated the majority of the enhanced semen smears as a 1 meaning proteinaceous materials were visible after enhancement with the dye stains Amido Black and Hungarian Red and with the Zar-Pro™ Fluorescent Lifters. The fluorescent intensity was also rated under alternate lighting with Zar-Pro Fluorescent Lifters resulting in the highest fluorescence rating from the recovered semen smears. The dye-stains were effective as an insitu enhancement method, but the Gellifters did not effectively lift the stained smears. Even with the fluorogenic properties of Hungarian Red the stained smears on the black Gellifters were not readily visible under alternate lighting. As for the Zar-Pro™ lifted impressions the semen smears were not visible under normal lighting but were readily visible and brightly fluorescent under alternate lighting. It can be concluded that semen will enhance similar to blood thus if a semen impression was deposited on decedent skin it would be enhanced the same as a blood impression through the early stages of decomposition.

The blood impression collected in the fetal pig trials also produced similar results to the human decedent trials but unlike in the preserved pig trials, the fresh pig skin behaved more like typical human skin. The preserved pig skin was more porous and spongier in comparison to the fresh skin, soaking up more of the dye stain than the fresh pig skin and even reducing the proteinaceous materials on the skin which affected visualization of the blood and limited bonding with the Gellifters and Zar-Pro™ Fluorescent Lifters. Through most of the nine-day collection intervals the blood impressions on the fresh and preserved fetal pig skin were notably visible, even if faint detection was not a significant concern. However, as expected detection of the blood on the preserved skin was more difficult than on fresh pig skin. Amido Black and Hungarian Red were effective enhancement methods for insitu dye-staining through the five-day collection intervals, with both stains having limited success through the nine-day collection intervals. The limited success in the fresh pig trials was in part due to precipitation which adversely affected enhancement

after the five-day collection interval. Gellifters were not overly effective for the recovery of the dye-stained impressions in either the preserved or fresh pig trials. Even with the fluorescent properties of the Hungarian Red dye-stain, the fluorescent intensity was not significant enough to improve the visualization of impression details. The Zar-Pro™ Fluorescent Lifters were able to effectively bond to the proteinaceous materials in the blood impressions which were not always readily visible under normal lighting for the preserved pig trials, but impression details were visible under alternate lighting through most of the nine-day collection intervals. In the fresh pig trials, the Zar-Pro™ lifted impressions were visible under both normal and alternate lighting through most of the five-day collection intervals, however due to precipitation, impression details were lost through the remaining nine-days. Cohen's Kappa statistical assessment of examiner ratings determined almost perfect agreement between the two student examiners for both impression details and fluorescent intensity ratings. Thus, the examiners had significant agreement amongst the enhancement effectiveness for the recovery of blood impressions from the fetal pig skin. Fresh pigs had less variability than the preserved pigs indicating fresh pig skin is a better proxy to human skin and future studies should use fresh pig skin for research purposes.

The condition of the pigs and impressions were compared to the data sets to examine correlations between the weather conditions and the quality of impressions recovery at each collection interval during the early stages of decomposition. Throughout the duration of the trials, the preserved and fresh fetal pigs did not have any substantial insect activity and the skin stayed intact for the nine-day duration of the trials, thus future trials could continue past nine-day collections for fetal pigs. The preservation of the pig skin through nine-days may have been in part due to the colder weather during the seasonal fall trials. The environmental factor that had the most significant impact on the ability to enhance and recover the blood impressions from fetal pig skin was precipitation. The preserved pigs were exposed to rainfall prior to the two-day collection interval and then again at the three-day interval however it wasn't until the six-day collection interval that the preserved pigs were significantly affected with moisture accumulating on the skin. The fresh pig trials were also affected by precipitation at the five and six-day collection intervals with moisture accumulating on the skin. Moisture on the skin adversely affected the ability of the dye-stains to bond to the proteinaceous impressions and created smearing and smudging on the Zar-Pro™ Lifters due to the moisture smearing the blood during the lifting process. Thus, precipitation in the form of rain or snowfall was concluded to be the environmental condition that affected the ratings most during both trials. The ratings of the impression details decreased as soon as precipitation was noted with accumulated moisture on the skin, around the five-day collection intervals for both the preserved and fresh pig trials.

During the trials environmental and anthropological variables were recorded and assessed to determine the environmental significance on the recovery of impressions from skin, as this area of research has yet to be thoroughly studied. Impression recovery from the skin during the early stages of decomposition will be affected by anthropological and environmental variables as decedents progress through the early stages of decomposition. Other than precipitation these variables, however, do not by themselves prevent the recovery of blood impressions or semen smears from skin, even over the course of several days. Throughout the trials it was determined that blood impressions are not stable on the skin substrate until they remain insitu from anywhere between twelve to twenty-four hours. It appears that the longer the blood remains insitu on skin increases the stability of the impressions, thus impressions aged insitu are not as easily destroyed from precipitation. The force at which the precipitation contacts the impression area, as well as the distance from the impression to the water source will affect the condition of the impressions. Even a

heavy mist if not contacting the impression areas with a strong force will not have a detrimental effect on the integrity of the blood impressions, thus wet impressions can still have viable impression details even under pooled water. A simple method of drying a wet impression with a hairdryer prior to lifting was tested, and the resulting lifted impressions were analyzed to evaluate the efficacy of the drying method. As a result of these trials, it was concluded blood impressions affected by environmental precipitation should be dried to eradicate moisture from the impression area prior to lifting with Zar-Pro™ Fluorescent Lifters. As the research trials indicated drying can be done with the use of a blow dryer to improve the effectiveness of the Lifters, as well as the overall quality of the lifted impression, thus an improved analysis could be performed.

The success of the research trials was in part due to the recovery of the blood impression and semen smears by lifting impressions from a substrate onto the Gellifters of Zar-Pro™ which helps to remove substrate variables which can hinder the visualization of impression details. Skin has many substrate variables, first off it is a semi-porous substrate which can be problematic for insitu dye staining as the liquid stains will absorb into the skin substrate creating distracting background staining. Other variables of skin are dermal textures, such as creases, scars, hairs, and dermal pattens such as moles, freckles, or liver spots, many of which are darker colored than the skin and can mimic the fine details present in impressions making visualization of impressions insitu on skin more difficult. Zar-Pro™ Fluorescent Lifters were the most effective enhancement method in the trials, due primarily to their fluorogenic properties but also because they lift the proteinaceous impression from the decedent skin onto the white background of the Lifter for visualization under normal lighting. If the lifted impressions were not visible under normal lighting, the combination of the proteinaceous materials affixed in close proximity to metals embedded in the Lifters excite fluorescent when visualized under alternate lighting. The fluorescent impression can then be visualized on the now darkened background of the Lifter, which again produces an optimal contrast for visualization of impression details. As the Gellifters were not able to effectively lift the dye-stained impressions, the use of Amido Black and Hungarian Red were limited to insitu enhancement methods. Only insitu enhancement followed by the use of Gellifters were done in the early collection intervals for the decedent trials as the removal of the epidermis would alter the integrity of the skin for the duration of the research. On the six-day collection interval, the epidermal skin was so fragile that the use of Gellifters were damaging the skin during lifting. As the skin was already being damaged in the lifting process, the dye-stained impressions were removed. This can be easily done during the later stages of early decomposition as the epidermal and dermal skin layers naturally separate. The removal of the epidermal skin containing the stained impression allows for improved visualization of impression details and helps preserve the impression long term while also improving the quality of insitu photography which can now be conducted in the controlled setting of the laboratory and not just in the field.

In many violent criminal interactions there is bloodshed, yet blood-based impressions are not a frequent focus of research and the constant substrate present in the involvement of violent crimes between two or more persons is skin, which is one of the least studied substrates. This study sought to understand the detection of this valuable evidence on human skin and to optimize enhancement methods to improve impression recovery throughout the early stages of decomposition. Not many studies have been conducted on this important substrate, human skin, with the results of this research being shared with the project collaborators at the Wayne County Medical Examiner's Office (WCMEO) to review for field-testing and eventual adoption for use in medico-legal cases. Thus, this study has the potential to directly influence standard practice in law enforcement.

Main Body

I. Introduction

Statement of the Problem

Blood and other proteinaceous impression evidence have great value in criminal investigations and can be present on human skin due to the nature of contact between people during violent physical encounters, such as bloody fingermarks around the neck of a choked victim or a semen impression on the thigh of a victim of a sexual assault. These types of interactions also apply to postmortem activities with impressions present on decedent skin in relation to moving or changing positions of a body. The constant substrate involved in the interaction between people during the commission of violent crimes is human skin. However, skin is one of the least studied substrates in the impression discipline and it has been stated that human skin is "perhaps the most difficult and unpredictable substrate for fingerprints that one can encounter" [12]. Another reason for the lack of knowledge in this area is due to the misconception that impressions on human skin are fragile and cannot be preserved after the original deposition. It was once estimated that the chances of recovering an identifiable print on human skin was 15,000,000 to 1[50]. In 2013, authors Lee and Gaensslen considered the inability to enhance and preserve impressions on human skin as one of the most difficult problems facing forensic scientists [32], yet there has been little research to improve the state of knowledge in this area.

Zar-Pro™ Fluorescent Lifters are a fairly novel enhancement method used to effectively lift, enhance, and preserve blood [16, 27, 65, 66, 67, 68, 70], semen [67, 68], and some saliva impressions from various substrates [67, 68]. Proteinaceous blood and semen impressions are long lasting and have been recovered from substrates even after being aged in situ for one-year [67, 68]. Zar-Pro™ Fluorescent Lifters have been used to effectively recover latent blood impressions after remaining in situ for five days on living human skin [70] and on decedent skin during the early stages of decomposition [Unpublished Data].

Amido Black is a commonly utilized chemical enhancement method for staining blood impressions on skin [7, 13, 14, 15, 30, 45, 51] and it has been used on decedent skin in case work [11, 14, 26, 28, 30]. Hungarian Red has also been utilized to enhance blood impressions on human skin [13] and has been recognized as a more effective method over Amido Black [45] and when enhanced impression are lifted onto white BVDA Gellifters® the impressions have fluorescent properties [15, 44, 61] to improve visualization. A comparative analysis between the novel Zar-Pro™ Fluorescent Lifters, and two known dye stains: Amido Black and Hungarian Red for the enhancement of proteinaceous impressions from human decedents had yet to be conducted and was the basis for the research project. These three methods were selected for use based on past-use and effectiveness, along with affordability and ease and safety of use at crime scenes.

Semen can also be enhanced using proteinaceous dye stains Hungarian Red and Amido Black [Unpublished Data], along with the use of Zar-Pro™ Fluorescent Lifters [67, 68]. Semen impressions along with semen smears may also be present on skin but are often overlooked due to their latent appearance thus, this vital evidence in criminal sexual assault cases, amongst others may not be recovered. This is unfortunate, as semen is long-lasting, like blood and can be recovered even after remaining in-situ on a substrate for a year [67, 68]. The latent appearance of even fresh semen stains limits their ability for detection, which is unfortunate as semen is long-lasting, therefore the ability to recover this valuable evidence is likely high.

The significance of environmental factors on the recovery of impressions from skin was addressed by Sampson and Sampson [51] but has yet to be thoroughly studied. Thus, the project sought to understand the effect of environmental and anthropological variables on blood impression recovery from human decedent skin during the early stage of decomposition. The effectiveness of the enhancement methods through the early stages of decomposition may result in the inability to recover impressions due to degradation and damage and this threshold of use is also a meaningful part of this project. Expanding forensic practitioner understanding of aged, degraded limited, damaged, or otherwise compromised physical evidence, specifically the enhancement and recovery of blood impressions and semen smears from decedent skin during the early stages of decomposition is a worthwhile research endeavor.

Literature Review

The three types of chemical enhancement methods generally used for blood are protein-reactive reagents, amino acid reagents, and peroxidase reagents. Protein-reactive reagents are more effective than peroxidase-reactive methods because they target proteins in blood and globular proteins in hemoglobin [31]. These methods include Amido Black, Coomassie Blue, Crowles Double Stain (combination of Coomassie Blue and Acid Red 71), Acid Violet 17, Acid Yellow 7, and Hungarian Red. Amino acid reagents Ninhydrin, DFO, and 1,2- Indanedione react with blood and are particularly effective on porous substrates. Peroxidase reagents are Benzidine, O-Tolidine, Diaminobenzidine, Luminol, ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulfonate]diammonium salt), Merbromin, Leuchorhodamine (LeuR6G), Leucocrystal Violet (LCV), Leucomalachite Green (LMG), and Fluorescein. Despite the large number of available chemicals, enhancement methods are limited in effectiveness based on substrate porosity, coloration, texture, fluorescence, background interference, and toxicity.

Enhancement method suitable for non-porous substrates will likely be inferior for porous substrates. Amido Black [8, 20, 31, 35, 39, 40, 41, 54, 61], Coomassie [8, 18, 31, 35, 53, 61], Crowles Double Stain [20, 31], Acid Yellow 7 [8, 41, 52, 53], Acid Violet 17 [8, 31, 39, 53], Hungarian Red [8, 20, 41, 53, 61], and LCV [39, 40, 52, 61] are generally most effective for enhancing blood impressions on non-porous substrates due to background staining, which makes visualization of ridge detail difficult. Although, Amido Black has reportedly also been effective on porous substrates [30]. Ninhydrin [40, 52, 61] and DFO [39, 52, 61] are generally more effective for porous substrates but not particularly useful on non-porous ones. There are some chemicals that create fluorescent impressions, such as Hungarian Red [8, 15, 44, 31, 61], Acid Yellow [8, 15, 31], Diaminobenzidine [49], Fluorescein [31], Merbromin [59], LeuR6G [18, 62], LCV [15, 30, 57] DFO [61], and Luminol [18]. However, while Acid Yellow is effective on faint blood volumes, fluorescence is quenched as blood volume increases limiting its overall effectiveness [31]. Merbromin [61] and LeuR6G [18] are fluorescent, but like Fluorescein [31] produce distracting background interference obstructing visualization of impression details. Hungarian Red [15, 61], Ninhydrin [52], LCV [59], and Diaminobenzidine [49] exhibit fluorescence, but not with the sensitivity of the other methods, and visualization may be hampered by background interference. In addition, Luminol, although very sensitive to blood will destroy/degrade impression details [31]. Fluorescein is sensitive to blood and is a safer alternative than Luminol, but it degrades rapidly, developing background interference in only minutes [31]. Peroxidase-reagents are generally more reactive than protein-reactive reagents when working with decreased blood volumes, however, large blood volumes may result in quenched fluorescence [52, 53, 61], which limits the visibility of impression details.

Chemical toxicity, along with ease of preparation and application, determines whether the method is safe and/or practical for crime scene use. Benzidine [8, 31, 61, 64], O-Tolidine [7, 14, 31, 62], Diaminobenzidine [8, 31, 41, 61], and LMG [17] are known carcinogens and pose serious health and safety concerns, which has led to a ban on their use as enhancement chemical. The working solutions for Acid Yellow [52], LeuR6G [64], DFO [61], and Ninhydrin [61] are flammable, and Merbromin [49, 61] and LCV [4] are toxic. LCV is a known biohazard with the ability to damage human chromosomes [4]. Methanol is also considered toxic [44, 35, 61], thus methanol based Amido Black is not safe for crime scene use as with many of the above-mentioned enhancement methods. Water-based Amido Black [15, 35, 52, 53] and Hungarian Red [45, 53], which is also water-based have a low health hazard, thus both methods are considered suitable and safe for crime scene use.

Enhancement methods for impressions on human skin have been mentioned in literature since the 1930's [12] and were adopted for casework in the 1970's [12, 51]. A common misconception surrounding this form of evidence has maintained that impressions are not present on human skin and thus cannot be recovered. Even though, sporadic reporting on this topic suggests otherwise. Coomassie Blue [14], Acid Yellow [14, 15], LCV [1, 13, 15, 38, 45], Benzidine [39], O-Tolidine [3, 7, 10, 14, 62], and LMG [24] have all been applied to impressions on skin, with Amido Black as the most effective method [7, 30, 51]. Amido Black has also been used on decedent skin in case work [3, 11, 14, 26, 28, 30] and in one case, a 1993 Florida homicide, an Amido Black enhanced impression on the victim's skin was matched to a suspect who later confessed to the murder, along with three other homicides of women in the area [28].

The staining process for Amido Black can alter the skin appearance (blue/purple discolorations) [7, 15, 30, 41], potentially concealing or mimicking bruising or trauma, which may interfere with the autopsy findings [7]. Amido Black is the most widely accepted enhancement method for blood due to its ease of use [7, 15, 20] and strong contrast [8, 15, 20] and has successfully been used to enhance blood impressions on human skin [14, 15]. Amido Black has two different formulations: methanol-based and water-based. Alcohol in methanol based Amido Black could potentially affect toxicological screenings on decedents [7] as alcohol can be absorbed by the body through skin. Regardless of safety, the water-based solution should be utilized in medicolegal cases so as to not alter toxicology screening for the decedent [15, 51]. A comparative analysis between the methanol and water-based formulas of Amido Black have shown that methanol-based produces a greater contrast for visualization and is often preferred over the water-based formula [8, 35, 53] but both methods are effective [15, 51, 53]. Recently, Hungarian Red was utilized to enhance blood impressions on skin [13] and was recognized as a more effective method over Amido Black and LCV [45]. This dye stain also uses a water-based formula, which does not contain alcohol to affect toxicological findings at autopsy, and is safe for crime scene use [15, 45]. In addition, Hungarian Red enhanced blood impressions lifted with a BVDA Gellifters® have fluorescent properties when visualized under alternate lighting [15, 44, 61]. Chemical enhancement with Amido Black and Hungarian Red have been shown to not affect subsequent DNA analysis [20, 21].

In applying chemical enhancement methods to human skin, unique variables associated with skin must be considered, as skin is a difficult substrate [48] in part due to its porosity, curvature, and dermal patterns/textures (scales, hairs, and wrinkles). Skin features can visually mimic ridge details, obscuring actual impression details and making it difficult to determine impression quality even when blood is readily visible. In general, impressions on skin are not readily visible, as once the heme responsible for the characteristic coloration of blood is lost, impressions will become latent despite being retained in situ [70, Unpublished Data]. The ability to isolate an impression by lifting

it from the skin helps to eliminate substrate variables that impede visualization of impression details, thus making the impression identifiable or not. The benefit of lifting chemically enhanced blood impressions has been recognized [8, 13] and methods such as LMG enhanced impressions lifted with photographic paper [25]; LCV enhanced impressions lifted with nylon membranes [25, 38], alginates [1], dental stone [1], polyvinylsiloxane [1], and Mikrosil [1] or adhesive tapes [55]; O-Tolidine enhanced impressions lifted with adhesive tapes [3], and even the general lifting of enhanced impressions using gel lifters [8] have demonstrated limited success. Hungarian Red enhanced impressions lifted with BVDA Gellifters® have been used to recover blood impressions and are fluorogenic [15, 44, 61], providing an improved contrast for visualization of impression details.

Unique to the above-mentioned chemically enhanced and lifted methods are the Zar-Pro™ Fluorescent Lifters. This novel method does not require any chemical enhancement prior to lifting proteinaceous impression, if blood impressions are pre-treated, the Lifters will not be effective [41]. Thus, the Lifters are spray activated prior to use with Zar-Pro™ Activator. The activated Zar-Pro™ Lifters are highly sensitive to proteins which bond with proteinaceous materials, lifting impressions from the substrate onto the white background of the Lifter for visualization, without competing substrate variables [16, 27, 65, 66, 67, 68, 70]. The Lifters have been used to effectively lift, enhance, and preserve blood and semen impressions across a broad range of substrates over the course of one-year [67, 68]. Visible or partially visible blood impressions can be seen on the white background of the Lifters, while latent impressions, due to the loss of coloration in the blood, can be visualized under alternate, providing a brightly fluorescent impression on the darkened background of the Lifters [16, 27, 65, 66, 67, 68, 70]. Fragile or perishable impressions are also stabilized upon contact with the Zar-Pro™ Lifters, preserving impressions [65, 66, 67, 68, 70] and DNA (time sensitive) [67] in a “fixed” state. Fluorogenic properties of the Lifters are not diminished with time and are available for repeat visualization (to date, for at least 13 years) [Unpublished Data].

Zar-Pro™ Fluorescent Lifters have also been used to recover blood impressions from living human skin [65, 66, 70] and can be lifted from various locations on the body: forearm [70] as well as the neck [65] forehead, and inner thigh [Unpublished Data]. The quality of the impressions was difficult to assess on skin due to competing background variables. Once the impressions were lifted from the substrates, the quality of the impression details were evident [65, 66, 70, Unpublished Data]. In one study [70], blood impressions were deposited onto a living subject’s forearm and left in situ to age for one-hour through five days. In most of the aged intervals, blood impressions were not readily visible on the skin of the subjects prior to lifting, whereas the lifted impressions showed ridge details in the majority of lifts recovered after the five-day interval [70]. In preliminary decedent skin research conducted in the Summer of 2018, researchers deposited 80 blood impressions and 20 semen impressions on various body locations: neck area, thighs, arms, and legs of two unclothed decedent donors. After depositing the blood and semen impressions, the donors were placed (supine position) in a secured outdoor field at the FROST facility to examine the possibility of enhancing and recovering these impressions during the early stages of decomposition (Five days). Decedent donors were protected by a mesh cage and were covered by a canopy. This study demonstrated the enduring nature of blood-based impressions, as impressions could be recovered with visible details under normal and alternate lighting conditions at the one hour and 24 hours intervals utilizing Zar-Pro™ Fluorescent Lifters. After 24 hours, blood impressions were less visible on decedent skin and by the 60-hour interval, only blood residue without impression details was visible and the skin itself was deteriorating because of natural decomposition. Preliminary data

has shown limited success in recovering semen impressions from decedent skin [Unpublished Data], indicating further optimization of deposition parameters is necessary.

Amido Black, Hungarian Red, white BVDA Gellifters®, and Zar-Pro™ Fluorescent Lifters have been successfully utilized in the recovery of blood impressions from skin, thus this project does not seek to validate but to conduct a comprehensive review of these methods while studying blood and semen impressions during human decomposition. There are four or five stages of decomposition for terrestrial surfaces and the rate at which a cadaver moves from one stage to another depends upon the local environment, particularly temperature, moisture, and insect access, as well the condition of the cadaver and scavenger access [23, 34, 42, 46, 47, 56, 60]. Changes in decedent skin variables due to decomposition as they affect the recovery of impressions have yet to be explored and could create variations in secretions, skin tone, skin patterns, color, porosity, and dermal surface topography. Decomposition may affect detection of impressions in situ on skin, even though the proteinaceous materials holding the impression details likely remain. Thus, important evidence may be left on the skin of decedents and this study plans to address this concern. The ability to recover impressions across seasonal and geographical areas is another important aspect of this study and the recovery of impressions from frozen or snow-covered bodies or even extreme heat should not be dismissed. In a 1977 Michigan homicide case, a partial impression was recovered from the skin of a 10-year-old girl that had been found frozen in a snowbank and likewise in a July 1985 Florida homicide case an impression was recovered from the leg of a child victim [12].

This project aims to develop a future-focused work force by providing students opportunities to participate in hands-on research across two institutions (Madonna University, and Northern Michigan University). This research will expand the capabilities of law enforcement to recover proteinaceous impressions that may be unknowingly overlooked or destroyed by practitioners due to visualization issues with skin. A common belief has been held that even with enhancement, the vast majority of latent impressions found on skin yield no prints of value for analysis [50]. Sampson and Sampson (2004) surveyed more than 4,000 of their "peers" and found only 1% had attended formal training courses on the recovery of print evidence from human skin and merely 12% had attempted to process a human body (alive or dead) for the presence of impression evidence. In addition, they concluded "more well-trained people are needed and body-processing should be attempted much more often than it has been" [51].

Rationale for Research

The goal of this project was to determine the effectiveness of enhancing blood and semen impressions utilizing cost effective, non-toxic methods that are safe for use at crimes scenes to provide opportunities for recovery of valuable impression evidence from decedent skin. As a result, a universal protocol for the collection of proteinaceous impressions from decedent skin will be developed in collaboration with the WCMEO and will be available to law enforcement agencies for review and adoption. To paraphrase a statement made by LaForte (2012), the chances of recovering fingerprints from human skin would be none if the body is not processed for such evidence [28].

The enhancement and preservation of impression evidence is directly applicable to forensic science and can be utilized to assist in identifying impressions on the skin of homicide or assault victims. Illustrating to members of the forensic science community that latent impressions are present on the skin of decedents and can be lifted, enhanced, and preserved for analysis with the possibility of obtaining a DNA profile would be a powerful tool for law enforcement and could potentially lead to suspect identifications. New knowledge and improved understanding on this topic will have a direct and immediate impact on forensic applications for medicolegal

investigations and will serve to guide criminal justice policy and practice in forensic science. An additional aim of this project was to help develop a future-focused workforce by involving student researchers from Madonna University in collaboration with student researchers at Northern Michigan University, Forensic Research Outdoor Station (FROST) facility, Marquette, Michigan, in total 24 students participated in this research project.

II. Research Methods

1. Personal Protection and Sanitation Practices

Research was conducted at the Madonna University Forensic Science Research Facility (MUFSRF) as well as the Forensic Anthropological Research Laboratory (FARL) and Forensic Research Outdoor Station (FROST) on the campus of Northern Michigan University. MUFSRF is in the LEED Gold Certified Franciscan Center for Science and Media Arts. The MUFSRF has separate labs for different types of forensic-related research and a secondary limited access laboratory used for storage. Researchers involved in the impression deposition and enhancement processes wore appropriate personal protective equipment (PPE) including Tyvek suit, footwear covers, eye protection, gloves, and mask. Gloves were always worn and changed every 20 minutes to prevent possible contamination. Any contaminated surface (e.g., gloved hand) was kept away from exposed skin to prevent contact with mucosal membranes (e.g., eyes, nose). All implements (e.g., forceps, scissors) were sterilized prior to and after use in the trials. Gloves were changed before lifting impressions from separate placement areas on the decedent to prevent cross contamination. After impression collection was complete, PPE was properly disposed in accordance with laboratory protocols.

2. Optimization of Blood Impressions and Semen Smears

Biological fluids for deposition

The biological fluids utilized in this study were research grade bovine blood (Innovative Research) or pre-screened human blood (Innovative Research) and pre-screened human semen (Innovative Research). Th human biological fluids were pre-screened for use according to the Food and Drug Administration (FDA) requirement with K2 EDTA anticoagulant added to the blood samples upon collection. The bovine and human blood were stored and refrigerated between uses in pre-aliquoted tubes at 4°C and the semen was stored and frozen at -20°C in pre-aliquoted tubes. Before depositions, blood and semen aliquots were warmed to 37°C, the average core body temperature, using a mini dry bath (Benchmark) and then vortexed with a Bench Mixer (Benchmark) to mix the contents. Bovine blood was utilized during the study design phase and fetal pig trials, whereas human blood and semen were used during the human decedent trials.

Optimization of impressions while controlling substrate variability

Deposition parameters for optimal-quality blood impressions were set for the substrates utilized in the study design phase, as well as the main substrate of the research which was fetal pig or human decedent skin. Reproducibility of impressions is difficult, making the optimization of deposition parameters essential to help create consistent and comparable impressions for analysis in research trials [69]. Optimal quality impressions with visible proteinaceous material and visible ridge detail; including the overall impression pattern, ridge paths and deviations, such as dots, ridge endings, and bifurcations were deposited on the substrates thus all impressions for the trials were

individualizing when deposited. Using optimal quality impressions in research allows for interpretation of the variables associated with the degradation of the impressions and not the numerous variables associated with the deposition that may affect impression quality [66, 70, Unpublished Data]. Semen impressions on human skin are more difficult to optimize, as they are latent thus the quality of the deposited impressions is not easily assessed. Due to this, smears were made in the deposited semen instead of an impression, thus leaving a semen smear to be enhanced and recovered in human decedent trials.

Trained student research assistants deposited optimal quality impressions onto the substrates in the study design phase and research trials. During the deposition of blood impressions in the study design phase and fetal pig trials efforts were made to keep the laboratory ambient temperature between 23-25°C to minimize deposition parameter variation amongst research trials. The fetal pigs were refrigerated (4°C) prior to use and warmed to 15-20° C, closer to the ambient laboratory temperature (around 23°C) in accordance with the MUFSRF facility and season. Whereas the human decedents were frozen (-4°C) for over eight months and then refrigerated (4°C) for twelve days before being returned to the freezer (-4°C) for two days prior to the start of human decedent trials. The decedent skin was already starting to decompose; thus, the decision was made to deposit the blood impressions and semen smears in the refrigerated laboratory (approximately 17°C) at FARL in an attempt to delay additional decomposition until field placement. The decedent skin ranged in temperature from 12.8-15.6°C during depositions, which was colder than the fetal pig trials. Thus, the temperature variation between the pig and human decedent skin could slightly affect impression deposition between the trials.

Optimal Deposition Parameters for Blood Impressions

In order to standardize the deposition of blood impression, each depositor calculated the surface area of their thumb and monitored their body temperature. The surface area of the depositor's thumb was calculated by measuring and multiplying the length and width of the friction skin, with depositors' thumbs ranging from 0.74 to 0.96 in^2. The surface area calculations allowed for the quantification of blood volume based on thumb size; if the surface area of a depositor's thumb increased, blood volume was also increased to get complete blood coverage on the friction skin. Blood volume ranged from 13µL to 20µL and was adjusted accordingly based on surface area and/or the temperature of the depositor's friction skin, which ranged from 26-35 °C for the fetal pig trials and 25-32°C for human decedents. The depositor's thumb temperature was difficult to regulate, thus minor adjustments were made continuously as individual body temperatures fluctuated. As a rule, if the depositor's body temperature increased blood volume also needs to be increased to create optimal quality impressions.

A blood volume around 18-20µL, as well as pre-and post-deposition waiting intervals were predetermined for the deposition of blood impressions on skin using living human subjects. The warmed blood was pipetted with a P20 pipette (Gilson) directly onto the depositor's thumbs from pre-aliquot tubes and dispersed evenly over the friction skin using their index finger. The pre-deposition waiting interval is the time in which the blood remained on the thumb until the impression was deposited onto the substrate. During this time, the depositor's thumb was held horizontally in anatomical position for approximately 30 seconds but again this time did fluctuate slightly depending on changes to the depositor's body temperature. Deposition pressure, which is the amount of pressure applied to the substrate when depositing the impression ranged from 8-10 lbs. The post-deposition waiting interval ranged from between 8 to 10 seconds, the time that the depositor's thumb remained in contact with the substrate while depositing the impression. A digital clock was utilized to record both the pre- and post-deposition waiting interval and the deposition

pressure interval, and a digital scale was utilized to determine the approximate deposition pressure. Past studies have shown these guidelines have helped create consistent and reproducible impressions for analysis [29, 66, 67, 68, 70]. Fingerprints are considered personal identifying information, so the depositor's fingerprint samples were collected with the informed consent of the individuals involved in this research study and in accordance with institutional standards.

Using the outlined parameters, optimal quality impressions were deposited on to skin for the research trials, meaning each deposited blood impression had visible proteinaceous material with visible ridge detail to include overall impression pattern, ridge paths and deviations. If the impression was not deemed optimal quality, it was removed with water and Q-tip and allowed to dry before a replacement impression was deposited in the designated area. All deposited blood impressions were marked with a half-circle using a permanent marker (Sharpie Brand) to assist in detecting the biofluids throughout the research trials, which may be valuable as the blood become latent during the early stages of decomposition.

Deposition of Semen Smears

Semen smears were made using 20µL of semen pipetted with a P20 pipette (Gilson) directly onto the decedents skin from pre-aliquot tubes. The semen deposited onto the skin was left to sit for 5 minutes before the depositor did a single swipe to create the semen smear. A digital clock was utilized to record the time between the deposition of the semen and the smearing of the stain. Semen smears are latent thus were not visible even at the time of deposition and were marked with a V-shape using a permanent marker (Sharpie Brand) to identify the designated deposition area.

3. Enhancement Methods

The protein dye stains: Hungarian Red (Tri-Tech Forensics) and Amido Black (Tri-Tech Forensics), and Zar-Pro™ Fluorescent Lifters (Tri-Tech Forensics) with Zar-Pro™ Activator were selected for use in the research trials due to enhancement effectiveness, affordability, and the fact that they are easy and safe for crime scene use.

Dye Stains: Hungarian Red and Amido Black

Hungarian Red and Amido Black are both non-toxic, aqueous (water-based) proteinaceous dye stains pre-made with fixative for use on proteinaceous impressions such as blood and semen. The dye stains are used with an associated de-staining solution used to remove excess dye stain to improve impression visualization. Both dye stains and de-staining solutions were used according to manufacturer's directions.

Development of an Insitu Staining Enhancement Tray

A prototype enhancement tray was developed in the study design phase, this tray helped prevent unnecessary staining outside the impression area when using liquid dye stains: Amido Black and Hungarian Red on fetal pig and human decedent skin. Due to the proximity of the blood impressions deposited on skin in the research trials, the tray was necessary to optimize the available substrate, especially as only a limited number of decedents were available for research.

Several prototype trays were developed and tested in a series of trials with the chosen enhancement tray producing superior results. The enhancement tray was made from a small (1.61x1.61x0.31 inch) plastic weigh boat (Pure Ponta, Amazon), vinyl foam tapes (Frost King Brand), silicone sealant (Clear, Loctite Brand), adhesive latex (Clear, Creature Brand), and super plus tampons (Super Plus Absorbency, Tampax Brand). A hole was cut into the top of the weigh

boat using a scalpel (Figure 1a) for access to apply the liquid dye stain to the impression area. The small weigh boat was selected as it was larger enough to fit around a thumbprint without affecting the surrounding areas of skin. Foam was placed on the four edges of the weigh boat to allow for the tray to mold to various contours of a body. Silicone sealant was applied to the foam tape using a 20ml plastic syringe to create a ¼ inch thick layer of sealant around the edge of the tray as a waterproof barrier. Lastly, super plus tampons were cut in half and then attached to silicone with adhesive latex to act as an absorption barrier for excess liquid on the bottom of the tray (Figure 1b). The trays (Figure 1) are cost-effective and easy to assemble with a total of 500 trays pre-assembled prior to the start of the fetal pig and human decedent trials using materials that are non-toxic and safe for use at crime scenes.

a) Top view ***b) Bottom view***

Figure 1: Enhancement Tray

To test the functionality of the chosen tray for use in the fetal pig and human decedent trials, a series of tests were conducted on the skin of living human subjects, specifically on arms, legs, neck, and the upper chest/clavicle area. A thumbprint left in black fingerprint powder and affixed to the sticky side of tape was stuck to the body areas of focus to indicate the impression area. The tray was then placed atop the impression area with the tampon side firmly pressed to the skin and secured into place with tape (3M Transpore Medical Tape). Using a disposable pipette water was pipetted in small increments into the Enhancement Tray to determine if there was leaking, the tray was successful in holding 5mL of water reliably for 2 minutes. Ultimately the enhancement tray was determined to be suitable at preventing staining outside the impression area.

Lifting Stained Impressions with BVDA Gellifters®

During the study design phase, black and white BVDA Gellifters® were assessed for their effectiveness in lifting Amido Black and Hungarian Red stained blood impressions from semiporous substrates. The low-adhesive gelatin layer of the Gellifters bonds with the dye stain, lifting a mirror image copy of the impression onto the white or black background of the lifter for visualization and preservation. Often, the choice of the lifter color (black or white) is selected based on the color of the dye stain utilized in the enhancement process to produce an optimal contrast for visualization under both normal and alternate lighting. To determine whether black or white Gellifters would be used to lift the Hungarian Red or Amido Black stained impressions a series of tests were conducted in the study design phase.

Semi-porous substrates: white gloss photopaper (Office Depot) and tan craft foam (Dollar Store) were selected for this study, as skin is also a semi-porous substrate. Blood impressions were deposited on test substrates and then stained with Hungarian Red and Amido Black. After one minute the impressions were destained again according to the manufacturer's instructions. Amido Black lifted impressions were readily visible on the white Gellifters but not as visible on the black

Gellifters under normal lighting conditions, as the dye stain is a blackish blue color. When the Amido black lifted impressions were visualized under alternate lighting (455nm or 505nm with various barrier filters: red, orange, and yellow) on the white Gellifters impression details were visible but not fluorescent thus the use of alternate lighting for Amido Black lifted impressions was not deemed beneficial for visualization. As a result, it was determined that Amido Black stained impressions will be lifted with white BVDA Gellifters® for the duration of the research trials and only photographed under normal lighting conditions. Hungarian Red stained impressions were visible under normal lighting conditions on both the white and black Gellifters with white Gellifters providing the best contrasting background for visualization of the fuchsia-colored stained impressions. However, Hungarian Red is a known fluorogenic dye stain, and the lifted stained impressions are fluorescent when visualized under alternate lighting conditions (455nm or 505nm with various barrier filters: red, orange, and yellow) on both white and black Gellifters. The dark colored, black Gellifters provided the best contrasting impression when visualized under alternate lighting. Thus, it was determined Hungarian Red stained impressions will be lifted with black BVDA Gellifters® for the duration of the research trials and photographed under both normal and alternate lighting conditions (505nm with an orange barrier filter).

Optimization of BVDA Gellifters® to Decrease Smudging and Smearing

In addition to making a color determination for the use of white and black Gellifters for each dye stain, the time in which the impression was left stained on the substrate prior to lifting was quantified to optimize impression recovery methods. Blood impressions were deposited on semi-porous substrates: white gloss photopaper (Office Depot) and tan craft foam (Dollar Store) then stained and destained using Hungarian Red and Amido Black before being left insitu for the following intervals: 0, 10, 30, 60, 120, and 180 seconds before lifting with a white BVDA Gellifters. Only white lifters were used in these tests as impression details were readily visible under normal lighting. The Hungarian Red stained impressions lifted better than Amido Black stained impressions but with both methods there was still a significant amount of smudging and smearing in the lifting process.

Amido black stained impressions worked best when lifted 0-10 seconds after staining, allowing the transfer of the dye-stained impression onto the Gellifters. If the impression was not lifted right away none of the dye was transferred. This timeframe was tested on white gloss photopaper and tan craft foam. The foam substrate was more porous than the glossy paper thus there was more smudging and smearing of impression details during the lifting process with the foam hypothesized to be more similar to the conditions of skin. Hungarian Red stained impressions worked best when lifted around 120 seconds after staining. If lifted before the stain could dry 0-60 seconds, the impression details were more likely to be smeared or smudged when transferred onto the Gellifters and if lifted at 180 seconds the dye was too dry and impression details were not transferred which was evident in the glossy paper and tan craft foam trials. The craft foam which was deemed more similar to skin often had impression details smudged and smeared during the transfer to the white Gellifter even when waiting the 120 seconds after staining before lifting the dye-stained impression.

Increasing Efficacy for Lifting by Heating BVDA Gellifters®

Dye stains are effective for the enhancement of proteinaceous impressions in situ on substrates, but the BVDA Gellifters® do not do a great job lifting a copy of the stained impression, especially as the substrate porosity increases. Thus, additional efforts were made to increase the efficacy of the Gellifters for use in the research trials by heating them with a hairdryer (Revlon RV417, 1600-watt, compact travel dryer) with a two-heat setting: low and high heat. The low heat setting was

determined to be 125 volts and was selected to heat the Gellifters to keep the intensity minimal to preserve the integrity of the gel layer while making the gel tackier to increase the lifting ability. The high heat setting was 250 volts was deemed too hot, as it was destructive to the gel layer of the lifter. A series of tests were conducted using a hairdryer to heat Gellifters at a distance of 30 inches for the following intervals: 30 seconds, 45 seconds, 60 seconds, and 10 minutes. After these tests it was determined that 30 seconds was enough time to make the Gellifters tacky, and the additional times, even up to the 10-minute range did not make the gel-layer any more taky than the 30 second interval thus 30 seconds was the time the remaining Gellifters were heated prior to use.

Blood impressions were deposited on white gloss photopaper (Office Depot), and tan craft foam (Dollar Store) then stained and destained using Hungarian Red and Amido Black before being left insitu for the optimal post staining intervals of 10 seconds for Amido Black and 120 seconds for Hungarian Red. After removing the protective plastic covering of the white Gellifters, they were cut in half diagonally with the top of the Gellifters heated and the bottom half used according to manufacturer's directions. The Gellifters were then placed on the stained impression, gel side down with even pressure applied for five seconds, taking care to prevent any horizontal movement on the substrate which could cause the impression to smear. Stained impression details were visible on the Gellifters with both the heated and non-heated Gellifters producing very similar results. The impression lifted with the heated Gellifters appearing slightly less smudged than the non-heated Gellifters, but not all smudging was eliminated using either method. The Hungarian Red and Amido Black lifted impressions with the heated Gellifters both appeared slightly darker than the non-heated side. Heated Gellifters may produce slightly better impressions than the non-heated Gellifters however, the results were not drastically different thus the addition of time and resources didn't justify the use of the blow dryer, so it was not utilized in the fetal pig or human decedent trials. All tests were reproduced to ensure lifting parameters were optimized, as lifting impressions in the research trials will increase the effectiveness of the enhancement methods. Lifting stained impressions with the Gellifters provide another possibility to visualize the dye-stained impression by removing subtracted variabilities associated with skin. Additionally, lifting the stained impressions allows for the collection of physical evidence for transportation back to the laboratory which is not possible with in situ staining alone.

Zar-Pro™ Fluorescent Lifters

Zar-Pro™ Fluorescent Lifters (Tri-Tech Forensics) are stand-alone enhancement method with fixatives and fluorogenic enhancement capabilities embedded into the Lifters themselves, thus they do not require any additional fixatives or staining solutions. Not only is it not recommended, but it is also detrimental to the effectiveness of the lifters to apply additional fixatives or staining solutions to any proteinaceous impression before using the Zar-Pro™ Fluorescent Lifters. Proper storage of the Lifter prior to use is paramount, care should be taken to protect the Lifters from damage, as un-activated Zar-Pro™ is susceptible to be bent or scratched. The Lifters should also only be handled with nitrile gloves, as latex gloves contain proteins which could contaminate (create fluorescence) on the Lifters, as the Lifters can pull the proteins from latex gloves.

To use, the Lifters are activated with Zar-Pro™ Activator (TriTech Forensics) and then applied directly to the impression, lifting the impression onto the white lifter for visualization and preservation. The commercially available products purchased for this study will be used according to manufacturer's instructions and as the name indicates lifted impressions are visible under normal and alternate lighting conditions (455nm or 505nm with an orange barrier filter). Once lifted and dried, the impressions are permanently fixed to the Lifters membrane, allowing for the visualization of proteinaceous materials which will fluoresce long-term when visualized under alternate lighting

(505nm with an orange barrier filter). Again, Zar-Pro™ Lifters as sensitive and will bond indiscriminately to protein sources which can contaminate the Lifters and may obstruct visualization of recovered blood impressions or semen smears when visualized under both normal and alternate lighting. Thus, when working with Zar-Pro™ Lifters care must be taken to prevent contamination from external proteinaceous sources, such as packaging envelopes with blue dye and most ink pens and markers. Pencils were therefore used to label all packaging in the research trials.

4. Application of Enhancement Methods for Research Trials

Three optimized enhancement methods were utilized in the research trials, designated as Enhancements A (EA), B (EB), and C (EC).

Enhancement A (EA): Amido Black lifted with white BVDA Gellifters®

Amido Black dye stain (2mL) was applied to the skin through the access area in the enhancement tray using a 2mL disposable pipette. The liquid dye stain should cover the impression area with the absorption layer of the tray helping to prevent unwanted staining outside of the selected impression area for each trial. After one-minute, de-stain solution (2mL) was applied with a clean pipette to remove the excess stain from the impression with the absorption (tampon) layer of the tray again helping to prevent de-staining solution from affecting the skin outside of the impression area. A Student Researcher held the Enhancement Tray tight to the skin while the liquid dye stain was pipetted onto the impression area (Figure 2a). The option to hold the tray to the skin instead of securing it to the fetal pig or decedent skin was made to prevent any possible adverse effects of the tape to the decomposing skin. After another 30 seconds, white BVDA Gellifters® were used to lift the stained, bluish black colored impression (Figure 2a). The BVDA Gellifters® were pre-cut with sterilized scissors into fingerprint size lifters (1x3 inch) from the larger purchased sheets (13x18 inch) and secured in an airtight plastic bag to prevent them from drying out prior to use. To use, the protective plastic was removed from the Gellifter which was then placed gel side down on to the impression area. Adequate pressure was applied for 20 seconds using the backside of a gloved hand to ensure that the Gellifter thoroughly bonded with the stained impression, lifting a mirror imaged copy of the bluish-black impression onto the white background of the Gellifter to ensure optimal contrast for visualization under normal lighting. To preserve the lifted impressions for subsequent analysis, the used Gellifters were stored in a clean white paper envelope.

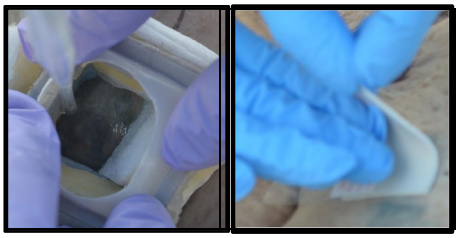
Enhancement B (EB): Hungarian Red lifted with black BVDA Gellifters®

Hungarian Red dye stain (1.5mL) was applied to the skin through the access area in the enhancement tray using a 2mL disposable pipette. The liquid dye stain should cover the impression area with the absorption layer of the tray helping to prevent unwanted staining outside of the selected impression area for each trial. After one-minute, de-stain solution (1.5mL) was applied with a clean pipette to remove the excess stain from the impression with the absorption (tampon) layer of the tray again helping to prevent de-staining solution from affecting the skin outside of the impression area. A Student Researcher held the Enhancement Tray tight to the skin while the liquid dye stain was pipetted onto the impression area (Figure 2b). The option to hold the tray to the skin instead of securing it to the fetal pig or decedent skin was made to prevent any possible adverse effects of the tape to the decomposing skin. After 120 seconds, black BVDA Gellifters® were used to lift the stained, fuchsia colored impression (Figure 2b). The BVDA Gellifters® were pre-cut with sterilized scissors into fingerprint size lifters (1x3 inch) from the larger purchased sheets (13x18 inch) and secured in an airtight plastic bag to prevent them from drying out prior to use. To use, the

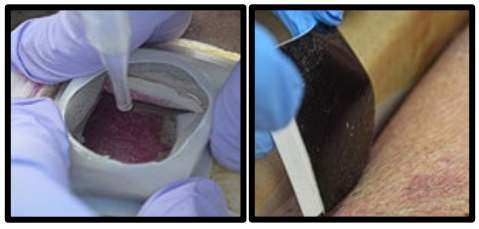
protective plastic was removed from the Gellifter which was then placed gel side down on to the impression area. Adequate pressure was applied for 20 seconds using the backside of a gloved hand to ensure that the Gellifter thoroughly bonded with the stained impression, lifting a mirror imaged copy of the fuchsia impression onto the black background of the Gellifter. The fuchsia color on the black Gellifter was not readily visible under normal lighting but due to the fluorescent capabilities of Hungarian Red an optimal contrast was achieved under alternate lighting (505nm with an orange barrier filter). To preserve the lifted impressions for subsequent analysis, the used Gellifters were stored in a clean white paper envelope.

Enhancement C (EC): Zar-Pro™ Fluorescent Lifters

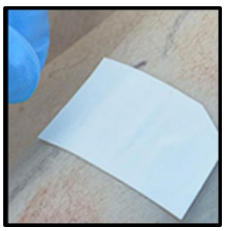
Prior to use, Zar-Pro™ Lifters were pre-cut with sterilized scissors into fingerprint size lifters (1x3 inch) from the larger purchased sheets (8x8 inch). Storage of the un-activated Zar-Pro™ Lifters is important, as un-activated Lifters are easily damaged from scratching or bending, therefore, after cutting the Lifters to size they were placed into a sterile plastic bag between two sheets of cardboard to prevent damage. The Lifters were then stored with the activation side labelled on the sterile plastic bag. Unlike the dye stains, Zar-Pro™ Lifters do not require a fixative or pre-enhancement staining prior to use. The Lifters are simply activated by spraying the Zar-Pro™ Activator onto the activation side of the Lifter until the Lifters are fully saturated. Excess activation solution must be evaporated from the Lifters before use to prevent the addition of pooled moisture onto the impression area. Once the Lifters are free from any pooled solution, they can then be applied to the impression area, activation side down (Figure 2c) with adequate pressure applied for 20 seconds using the backside of a gloved hand to ensure that the Lifter bonded with the proteinaceous impression. The lifted impressions were then permanently affixed onto the white background of the lifter for visualization under normal and alternate lighting (505nm with an orange barrier filter). To preserve the lifted impressions for subsequent analysis, the Zar-Pro™ Lifters were stored in a clean, dye free white paper envelope. It is important that the envelopes are free of dye, as the proteinaceous material within the dye-colored envelopes can contaminate the lifted impressions.



a)Amido Black/White Gellifter



b)Hungarian Red/Black Gellifter



c) Zar-Pro™ Lifter

Figure 2: Enhancement Methods

5. Detection and Documentation using Normal and Alternate Lighting

Careful examination of skin for biofluids, such as bloody fingerprints or semen smears were conducted to expand common knowledge amongst practitioners in the field on this topic. The presence of blood on skin can be readily detected under normal lighting due to the reddish-brown coloration presented for fresh blood stains. As blood ages the detection of blood impressions can be more difficult, which may also be compounded by potential changes occurring during the decomposition process. The postmortem changes of blood on skin have rarely been documented and is a focus of this study. Semen on the other hand is not readily visible under normal lighting conditions, even when fresh, but semen can be visualized under alternate lighting due to its

fluorescent properties. Biological fluids may obtain fluorescence for over 60 days, even after being thoroughly dried [59] with semen visualized under alternate lighting (405 to 505nm wavelength) best viewed with an orange or yellow barrier filter [58]. Even when biofluids are not fluorescent, alternate lighting can create noticeable contrast between the substrate and the biological fluid. Blood for example will generally darken under alternate lighting, making blood, especially latent blood more readily visible [58 and 59]. To aid in the detection of latent blood impressions and semen smears on skin during the early stages of decomposition an alternate light source (ALS) was utilized when impression details were not readily visible on the skin under normal lighting. In both the fetal pig and human decedent trials a Rofin Polilight® Flare Plus II was used with multiple wavelength (455 and 505nm) attachments and barrier filters (red, yellow, and orange). A black out forensic tent (Figure 3) was used in the field during the human decedent trials, when utilized the tent was placed directly over the decedent, which allowed for the use of an ALS during the daytime, as a dark environment is paramount to improve fluorescent visualization.

Throughout the trials photographs were taken under both normal and alternate lighting conditions. A Canon EOS T5i digital single lens reflex (SLR) camera fitted with an EF 100 mm Macro lens or a Nikon D5100 digital SLR camera fitted with an 18-55mm lens were used to document the research trials both in the field and in the laboratory. A Cannon speedlite 403 EX III-RT external flash was also available for use under normal lighting conditions depending on impression location and weather conditions in the field. When taking photographs under alternate lighting conditions, a handheld Rofin Polilight® Flare Plus II (505nm wavelength) was used to excite fluorescence which was visualized using a 62 mm orange barrier filter attachment for the camera lens. Overall, midrange, and close-up photographs were taken of the pigs and decedents prior to the deposition of blood impressions and semen smears in the laboratory, as well as throughout all collection intervals both before and after enhancement in the field. Close-up photos were taken of the impressions under normal lighting after the deposition of blood impressions and semen smears insitu on skin for the fetal pig and human decedent trials. Overall, midrange and close-up photographs were taken of each pig and decedent in their field placement both before and after every collection interval. Close-up photos were also taken in the field at each collection interval: prior to enhancement on the skin under normal lighting, insitu staining after enhancement for Amido Black (EA) and Hungarian Red (EB) under normal lighting. One hour after recovery of the dye-stained lifted impressions affixed to Gellifters (EA and EB) and on Zar-Pro™ (EC) under normal lighting and lifted impressions affixed to Gellifters (EB) and Zar-Pro™ (EC) under alternate lighting which were taken in the laboratory. Laboratory photography was conducted daily after each collection interval throughout the fetal pig and human decedent trials with the camera mounted to a stand for stabilization. For normal lighting photography, the camera settings were 1/40 of a second for shutter speed, f2.8 for aperture, and 200 ISO. For alternate lighting, the camera settings were 1/10 of a second for shutter speed, f5.6 for aperture, and 400 ISO with fluorescence generated by a handheld Rofin Polilight® Flare Plus II (505nm wavelength and orange barrier) with a fluorescence intensity scale included. One month after the field collections, a secondary set of the post enhancement lifted impressions were taken in the laboratory of the lifted impressions affixed to Gellifters (EA and EB) and on Zar-Pro™ (EC) under normal lighting and lifted impressions affixed to Gellifters (EB) and Zar-Pro™ (EC) under alternate lighting. All Photographs were taken with a photography scale labelled with the unique identifier specific to the trial, decedent, body location and enhancement method.

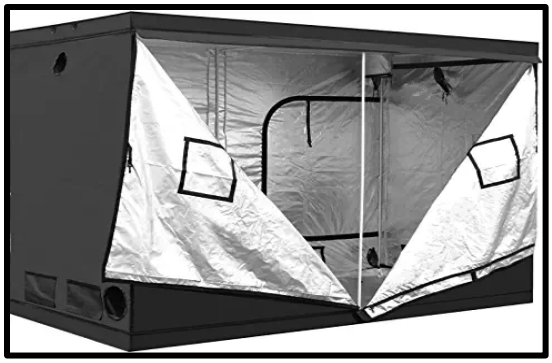


Figure 3: Portable Dark Room Tent

6. Transportation and Storage of Recovered Lifts

Recovered blood impressions and semen smears, meaning the Amido Black stained impressions lifted onto a white Gellifters (EA), Hungarian Red stained impressions lifted onto a black Gellifters (EB), and impressions lifted with Zar-Pro™ (EC) which were able to be preserved from the fetal pig and human decedent trials. These recovered lifts were stored in a clean, dye free white envelope that was pre-labelled with a unique identifier based on the trial, pig or decedent number, impression location, enhancement method, collection interval and sample number. Upon completion of the trials, recovered lifts along with archived photographs were transported from the field back to the MUFSRF for subsequent analysis. An inventory chain of custody log is being maintained to ensure sample integrity. The recovered lifts are stored in a locked file cabinet for long term storage and sample preservation in a secure facility at the MUFRSF.

7. Data management/Archiving

The analytical plan for data analysis outlined in this project resulted in a sizeable amount of raw digital data. The preserved fetal pigs resulted in 370 digital images for rating under alternate and normal lighting condition for both impression details and fluorescent intensity, resulting in 740 ratings assessed by two student examiners totaling 1,480 total ratings. The fresh fetal pigs resulted in 403 digital images for rating under alternate and normal lighting condition for both impression details and fluorescent intensity, resulting in 806 ratings assessed by two student examiners totaling 1,612 total ratings. The human trials for decedent donor 1 and 2 resulted in 1,283 digital images rated under alternate and normal lighting condition for both impression details and fluorescent intensity resulting in 2,566 total ratings which were then assessed by two student examiners and two practicing latent print analysts, totaling 11,112 ratings to include the addition of intra-examiner ratings. All digital images and examiner rating sheets are archived and stored on a desktop computer with files backed up onto an external hard drive. The computer is password protected and only authorized users are permitted access to the archived data. Digital images will be stored as image files and the raw data from the project will be stored in excel document files. Data sets will be made available to forensic researchers upon request through the National Archive of Criminal Justice Data (NACJD). The robust amount of data archived in this project will be stored in accordance with institutional data sustainability requirements, thus will be available beyond the proposed work for additional independent assessments.

III. Data Analysis

1. Compilation of Data Sets

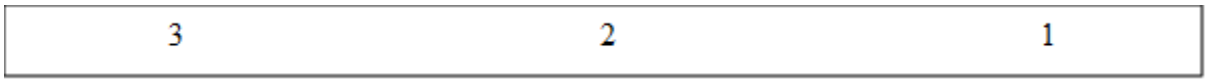
Data sets were compiled from the digital images taken over the course of two years in the fetal pig trials with the preserved fetal pig trials in the fall of 2020 and fresh fetal pig trials in the fall of 2021, as well as the human decedent trials taken during summer 2021. Pre-labelled digital images were archived and organized into data sets separated by trial, with each trial then separated by pig or decedent donor and collection interval. Due to the large file size of the digital images, the compiled data sets were posted on Dropbox with the links to each data set sent electronically via email for examiner assessment. The pig trials generated two primary data sets, one from the preserved pig trials and one from the fresh pig trials, which were then rated by two forensic science student examiners trained in impression analysis at Madonna University. The human decedent trials generated five data sets which were rated by two forensic science student examiners trained in impression analysis at Madonna University, as well as two practicing latent print analysts working on casework in American Society of Crime Lab Directors (ASCLD) accredited forensic science laboratories. A single data set was compiled from the semen smear trials rated by a forensic science student examiner trained in impression analysis at Madonna University.

2. Examiner Assessment

Examiners rated the digital images in each data set based on the quality of Impression Detail (ID) when photographed under normal and alternate lighting (505nm with orange barrier filter), as well the Fluorescent Intensity (FI) under alternate lighting (505nm with orange barrier filter) using a fluorescence scale standard developed by MUFSRF for use in research studies.

Impression Detail (ID) Rating Scale

Thumbprints deposited onto skin for the research trials were of optimal quality (ID rating 3; scale pictured below), thus the ability to monitor the degradation of the impression throughout the collection intervals as a result of decomposition and environmental factors could be assessed. Blood impressions remained insitu on the skin substrate with a subset of impressions enhanced at each of the collection intervals during the early stages of decomposition. The provided grey-scale impression standard was composed using ink to provide rudimentary guidance for impression quality that can be applied to the enhancement methods utilized in the research trials which were depicted in color. Using the archived digital images from the photographs taken during the research trials examiners rated the Impressions Details (ID) using the provided scale standard (Figure 4).

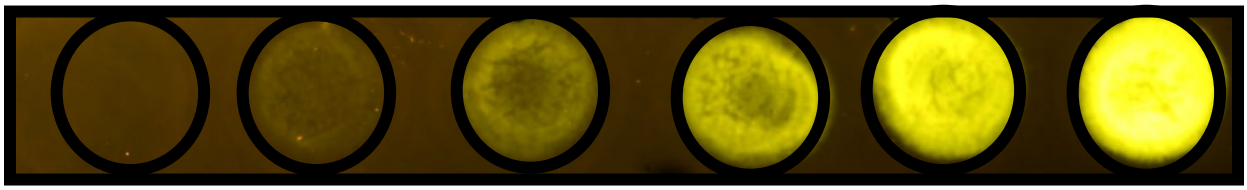


Impression Details (ID)
0 = no visible proteinaceous material, no visible ridge detail
1 = visible proteinaceous material, no visible ridge detail
2 = visible proteinaceous material, visible ridge detail;
such as a partial impression pattern with minimal ridge details
3 = visible proteinaceous material, visible ridge detail: overall impression pattern, ridge paths and deviations, such as enclosures, dots, ridge endings, bifurcations, etc.

Figure 4: Impression Detail Scale Standard

Fluorescent Intensity (FI) Scale

A solid phase fluorescence standard scale (scale pictured below) was developed to rate fluorescent intensity to alleviate common problems with quantifying visualization of fluorescence. Although it is recognized that the rating of fluorescence is somewhat subjective, the scale served as a standard for comparison throughout the trials. The scale was prepared by using serial dilutions of fluorescein in a 1% Photoflo™ and 10% methanol solution applied to Zar-Pro™ ™ Lifters in a linear array. A 4mg/ml stock solution of fluorescein was prepared and then diluted one part to one part in a series of two-fold dilutions. A seven-point rating scale was made using in 5µl drops from the following dilutions: 0.5 mg ml (highest- FI rating 6), 0.125mg/ml (FI rating 5), 0.0313mg/ml (FI rating 4), 0.00781mg/ml (FI rating 3), 0.00391mg/ml (FI rating 2), 0.00195mg/ml (lowest- FI rating 1). A zero rating would indicate no fluorescence and thus not featured on the scale. An ALS (Rofin Polilight Flare Plus II) at 505nm wavelength with an orange barrier filter must be used to excite and visualize the fluorescent scale (Figure 5).



1 2 3 4 5 6

Figure 5: Fluorescence Intensity Scale Standard

Using the compiled data sets examiners rated Impression Details (ID) and Fluorescence Intensity (FI) from digital images taken within an hour after the initial deposition in situ under normal lighting, and then for each collection interval photos were taken prior to enhancement in situ under normal lighting, dye-stained post-enhancement in situ under normal lighting, post enhancement lift under normal and alternate lighting, and a secondary set of digital images from the post enhancement lifts one month after collection under normal and alternate lighting.

Statistical Analysis of Examiner Ratings

To reduce any indirect subjectivity of research results intra- and inter-examiner variation were evaluated to protect the integrity of the project. Cohen's Kappa statistical analysis of inter- and intra-examiner ratings were applied to examiner assessments to verify the significance of the results

obtained. Inter- examiner variability shows differences amongst examiners whereas intra-examiner shows variability amongst the same examiner.

The following equation was used to find the level of agreement: $\mathrm{k} = \frac{\Pr(a)-\Pr(e)}{(1-\Pr(e))}$.

$Pr_{(a)}$ is the total number of ratings in agreement and $Pr_{(e)}$ is the total number of rating possibilities in each category. For normal lighting, the $Pr_{(e)}$ would 4 because there are 4 different possibilities to rate each normal lighting photo for impression details (ID 0-3). The $Pr_{(e)}$ for alternate lighting would be 4 for the different possibilities of impression details (ID 0-3) and 7 for possibilities of fluorescent intensity (FI 0-6). Therefore, the coefficient for the probability that one examiner would choose that rating for fluorescent intensity would be 1/7 which was the equivalent to 0.142 and the probability for impression detail would be 1/4 or 0.25. After that was calculated, the numbers could then be placed into the equation to be calculated.

To apply Cohen's Kappa to the fetal pig trials, two student examiners (Student Examiners A and B) rated the same data sets from both the preserved and fresh trials (inter-examiner variability) and their level of agreement was assessed. Their level of agreement was also measured from a subset of the original data sets and re-rated to measure the agreement to their previous ratings (intra-examiner variability). In the human decedent trials, two student examiners (Student Examiners 1 and 2) and two practicing latent print analysts (Examiners A and B) rated the same data sets. The level of agreement (intra-examiner) variability as well variability amongst their own ratings (intra-examiner) were assessed.

The strength of agreement amongst examiners was determined using the provided scale (Figure 6). The K value of the Cohens Kappa was calculated for each data set in the fetal pig and human decedent trials and compared to the provided scale to determine strength of agreement. Almost perfect or substantial agreement is indicative of agreement amongst examiners in the data set and amongst themselves. On the other hand, if there is poor or slight agreement then the reliability of results was not as substantial.

Cohen's Kappa statistic (κ)	Strength of agreement
< 0.00	Poor
0.00–0.20	Slight
0.20–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Substantial
0.81–1.00	Almost perfect

Figure 6: Cohen's Kappa Strength of Agreement

The statistical analysis will allow for a comparative assessment of the effectiveness of each enhancement method which will be considered along with cost, feasibility, and ease of crime scenes use for implementation into casework. The Wayne County Medical Examiner's Office (WCMEO) will be assessing the project results to review procedures for use in medicolegal cases in Wayne County, Michigan.

3. Assessment of environmental and anthropological variables

Weather and anthropological data were also assessed for the research trials. Weather data included ambient temperature and weather conditions in the field as well as temperature and

condition of the skin were recorded at each collection interval. An anthropological assessment was also conducted by an anthropologist at the FROST Facility during the human decedent trials which included the decedent donors Total Body Score (TBS). The TBS for each decedent was recorded throughout the collection intervals and was assessed along with the environmental data, insect & scavenger activity, and appearance of the donor remains to determine potential impact on the recovery of blood impression and semen smears from human skin using the determined enhancement methods.

IIII. Fetal Pig Trials

1. Intake and Preparation of Fetal Pigs

The purchased fetal pigs (Biology Products) were sexed, then weighed, and measured upon receiving at the MUFSRF. Six fetal pigs, triple injected with preservatives (identified as Preserved Pig 1- Pig 6) were utilized for the Fall 2020 trials and six fetal pigs, plain, un-injected (identified as Fresh Pig 1- Pig 6) were utilized for the Fall 2021 trials. The preserved pigs were chosen to ensure the skin was more apt to stay intact for the duration of the research trials, whereas the fresh pigs were preservative free. The preserved fetal pigs weighed an average of 4.89 pounds measuring between 12-14.5 inches in length, whereas the fresh fetal pigs weighed an average of 7.83 pounds measuring between 13.9- 15.35 inches in length. Prior to use, the pigs were washed with water and refrigerated (4°C), some of the pigs were also shaved with a disposable razor to remove the fine, white hair that covered the skin. This was done to allow for a better assessment of the enhancement methods, as hair is variant depending on the body location, thus had the potential to influence results.

2. Blood Impressions on Fetal Pig Skin

Three student research assistants deposited optimal quality impressions onto the skin of the fetal pigs for both the preserved and fresh pig trials. Each deposited impression was assessed to ensure optimal quality (ID rating 3), meaning visible proteinaceous material, visible ridge detail to include the overall impression pattern, ridge paths and deviations. In each trial, ten optimal quality blood impressions were deposited onto the pig skin in pre-designated area (left or right side of pig). The pigs were positioned for the trials either on their left or right side based on how they were packaged for shipment, it was found that the side of the fetal pig sitting in the liquid within the bottom of the plastic bag had a detrimental effect on the skin condition. Depositors rotated between the six pigs, so no single depositor was depositing all their impressions onto one pig. Thus, each depositor laid two impressions on each pig to diversify impressions for the trials. The forehead was left without any impressions, to serve as the area of the non-impression controls.

Sixty blood impressions were deposited for both the preserved (Figure 7) and fresh pig (Figure 8) trials with 10 impressions on each of the 12 pigs, totaling 120 impressions. The deposited blood impressions on the pig skin were marked with a half-circle using a black sharpie to ensure that if the impressions became latent the impression area could still be targeted for enhancement.

Figure 7: Preserved Pig 3 with Deposited Impressions

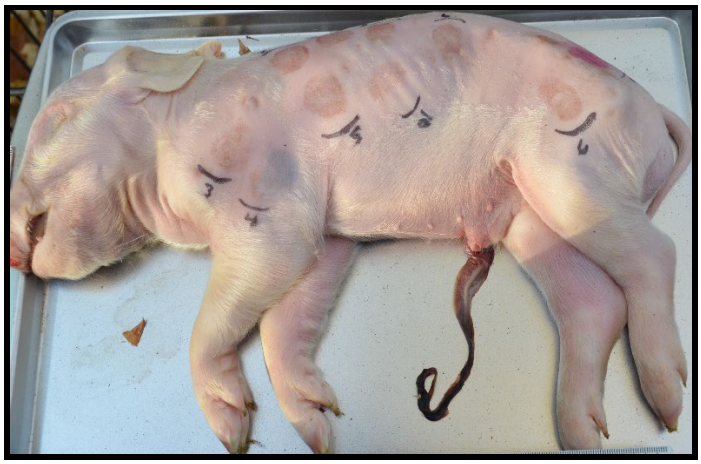


Figure 8: Fresh Pig with Deposited Impressions

3. Collection Intervals

An impression map (Appendix A; Figure 1) was developed for both the preserved and fresh pig trials with the blood impressions and non-impression controls given an identifying code indicating trial, pig number, impression location, collection interval, and enhancement method. The pigs were identified as 1-6 with the impression location of stomach (LS) and the non-impression control location of forehead (LF). Collection intervals ranged from the blood control (BC1), which were collected one-hour after deposition with a daily collection beginning at one-day (B01) and continuing through to the nine-day (B09) collection interval. One impression on the skin of each of the six pigs were enhanced at each collection interval (BC1, B01, B02, B03, B04, B05, B06, B07, B08, B09) utilizing the three-enhancement methods: Amido Black lifted with a white BVDA Gellifters® (EA), Hungarian Red lifted with a black BVDA Gellifters® (EB), and Zar-Pro™ Fluorescent Lifters (EC). Thus, two impressions were collected at each interval using EA, EB, and EC enhancement methods. Non-impression controls were collected at 1-hour (NC1), at 3-days (N03), and 5-days (N05) using Zar-Pro™ Fluorescent Lifters (ZP) and BVDA Gellifters® (GL). The non-impression controls were used to assess the effect of any inherent fluorescent properties associated with decomposition on the visualization of the recovered blood impressions.

4. Field Placement

After the 1-hour controls (BC1 and NC1) were enhanced and lifted in the controlled laboratory setting of the MUFSRF, the pigs were placed into a wooded field area on the campus of Madonna University for the remainder of the trials. The pigs were transported on metal trays lined with bench-coat and placed on top of pre-made wooden boxes to keep them off the ground, all of which were secured inside a large metal dog crate (Figures 9 and 10) and covered with a tarp (Figure 11). The crate served to protect the pigs from animal scavengers and the tarp helped to prevent direct rain or snowfall. After each enhancement interval, the pigs were rotated clockwise in the cage to ensure that each pig was subjected to similar environmental conditions throughout the trials.

Figure 9: Preserved Pigs Field Placement

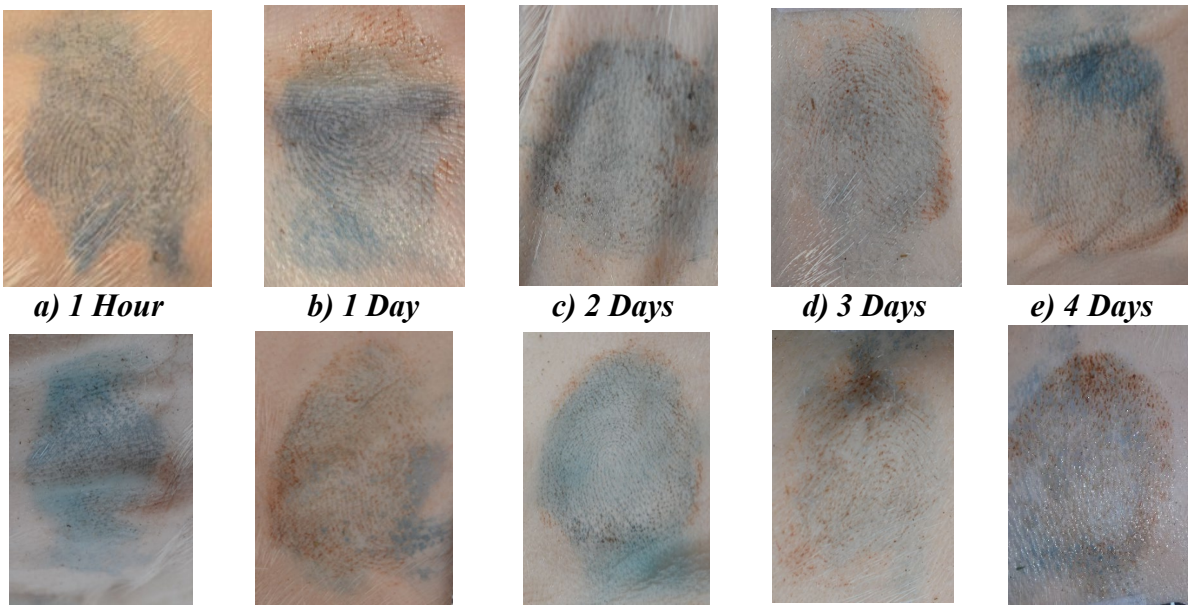
Figure 10: Fresh Pigs Field Placement

Figure 11: Tarped Cage

5. Results of Preserved Fetal Pig Trials- Fall 2020

Amido Black dye-stain was effective at enhancing blood impressions insitu on preserved pig skin at the one-hour (BC1) control interval (Figure 12a), allowing for the overall impression pattern and details to be visualized under normal lighting conditions. As the blood aged insitu on the skin, Amido Black continued to work but the staining was inconsistent amongst impressions throughout the nine-day (B09) collection interval (Figure 12b-j). The range of effectiveness may be due to variations in skin porosity, which may alter the way the stain was absorbed during the enhancement process, often creating very light stained impressions making it difficult to visualize impression details. The recovery of the dye-stained impressions through lifting with the BVDA Gellifter® was not effective, as lifting the stained impressions onto the Gellifter often resulted in a loss of impression details (Figures 13a-j) even at the control interval of only one-hour (BC1). The Gellifters did not effectively lift the Amido Black stained impressions with most of the lifted impressions having no visible ridge paths (Figures 13a, b, f, g, i, and j), while only a couple of the

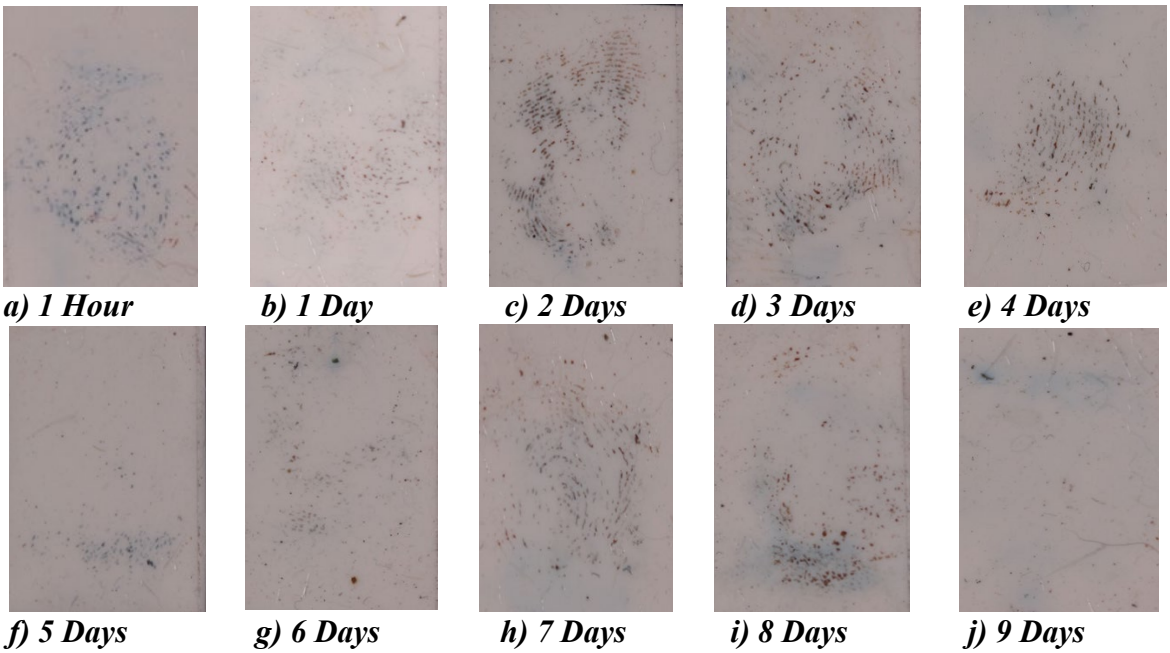
lifts produce partial impression patterns with ridge details (Figures 13c, d, e, and h). While the impressions were aged on the pig skin during decomposition, the impression quality decreased as expected with the nine-day (B09) collections not enhancing proteinaceous materials when visualized insitu after staining (Figure12j) or on the Gellifters ((Figure 13j). The dye-stained lifted impressions were photographed within one hour of lifting and then again one month later, it was determined that the Gellifters lost impression details between the one hour and one month timeframe thus the featured Gellifters (Figure 13) were all taken one hour after recovery.



a) 1 Hour ***b) 1 Day*** ***c) 2 Days*** ***d) 3 Days*** ***e) 4 Days***

f) 5 Days ***g) 6 Days*** ***h) 7 Days*** ***i) 8 Days*** ***j) 9 Days***

Figure 12: Insitu Amido Black Stained Blood Impressions

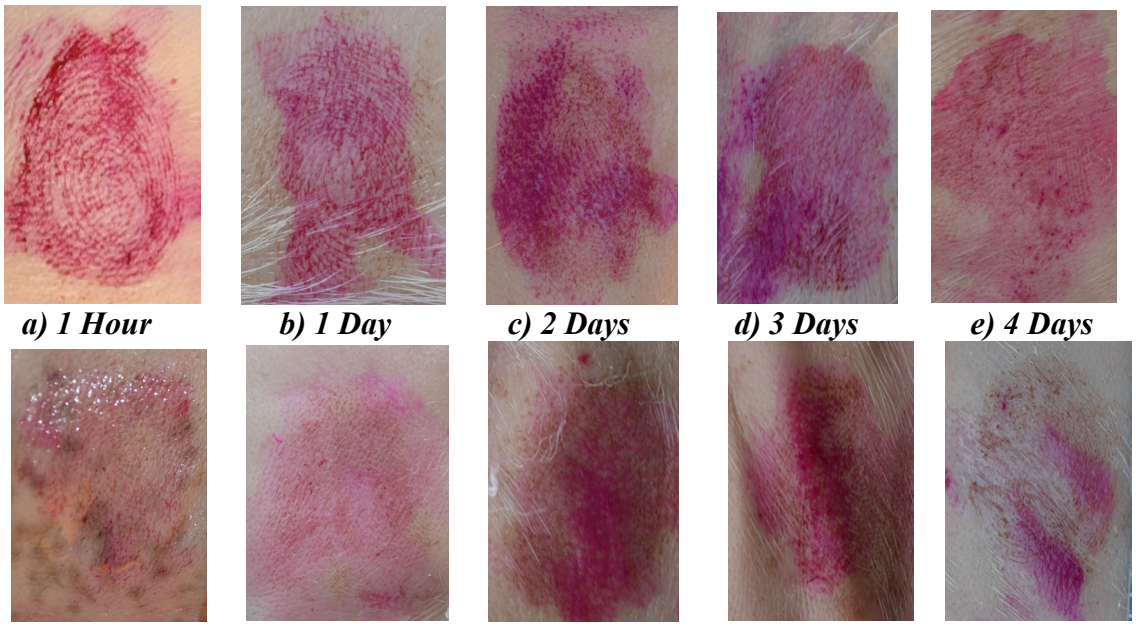


a) 1 Hour ***b) 1 Day*** ***c) 2 Days*** ***d) 3 Days*** ***e) 4 Days***

f) 5 Days ***g) 6 Days*** ***h) 7 Days*** ***i) 8 Days*** ***j) 9 Days***

Figure 13: Amido Black Stained Blood Impressions Lifted with White Gellifters

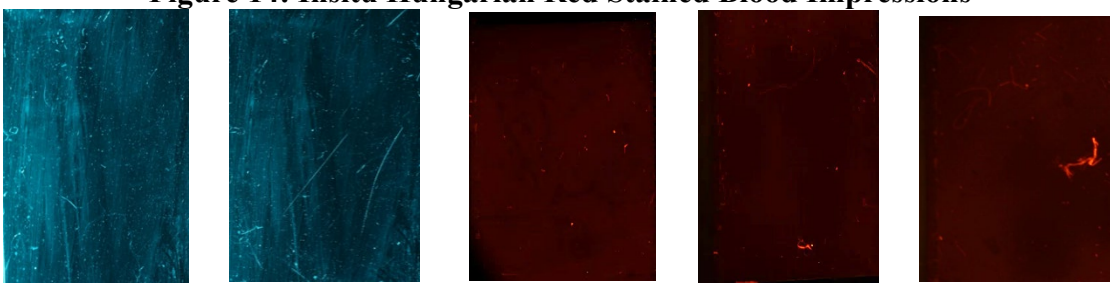
Hungarian Red dye-stain was also effective at enhancing blood impressions insitu on preserved pig skin at the one-hour control interval (Figure 14a) providing visible impression patterns with ridge details. The insitu dye-stained impressions were effective through the five-day (B05) collection interval, however there was a lot of inconsistency with the dye bonding to proteinaceous materials and or absorbing into the pig skin throughout the early stages of decomposition (Figure 14b-j). This was seen with the Amido Black stained impressions also and may be due to variation in skin porosity in different areas of the pig skin. The lifted dye-stained impressions were not readily visible on the black background of the Gellifters, even with the fuchsia color contrast under normal lighting conditions (Figure 15a and b). Due to the fluorescent properties of the Hungarian Red dye-stain the lifted impressions were also visualized on under alternate lighting in order to excite fluorescence of the dye-stained proteinaceous materials on the lifters. Weak fluorescence of the Hungarian Red stain was observed on the black background of the Gellifters, but no ridge details could be discerned (Figures 15c-j) and the most noticeable fluorescence was from collected cotton fibers from the enhancement trays (Figures 15e and h). The dye-stained lifted impressions were again photographed within one hour of lifting and then one month later, it was determined that the Gellifters lost impression details between the one hour and one month timeframe. The fluorescent properties of the dye stain did not improve with time either thus all the featured Gellifters (Figure 15) were taken one hour after recovery.



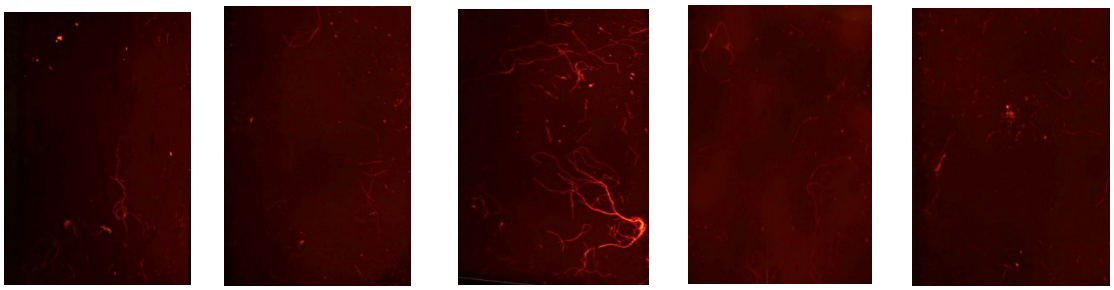
a) 1 Hour ***b) 1 Day*** ***c) 2 Days*** ***d) 3 Days*** ***e) 4 Days***

f) 5 Days ***g) 6 Days*** ***h) 7 Days*** ***i) 8 Days*** ***j) 9 Days***

Figure 14: Insitu Hungarian Red Stained Blood Impressions



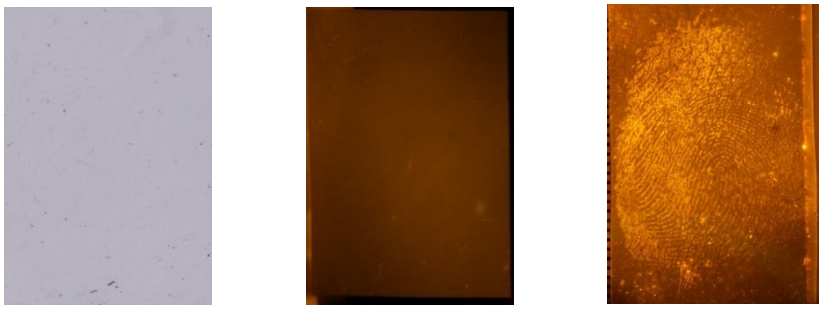
1 Hour ***b) 1 Day*** ***c) 2 Days*** ***d) 3 Days*** ***e) 4 Days***



f) 5 Days *g) 6 Days* *h) 7 Days* *i) 8 Days* *j) 9 Days*

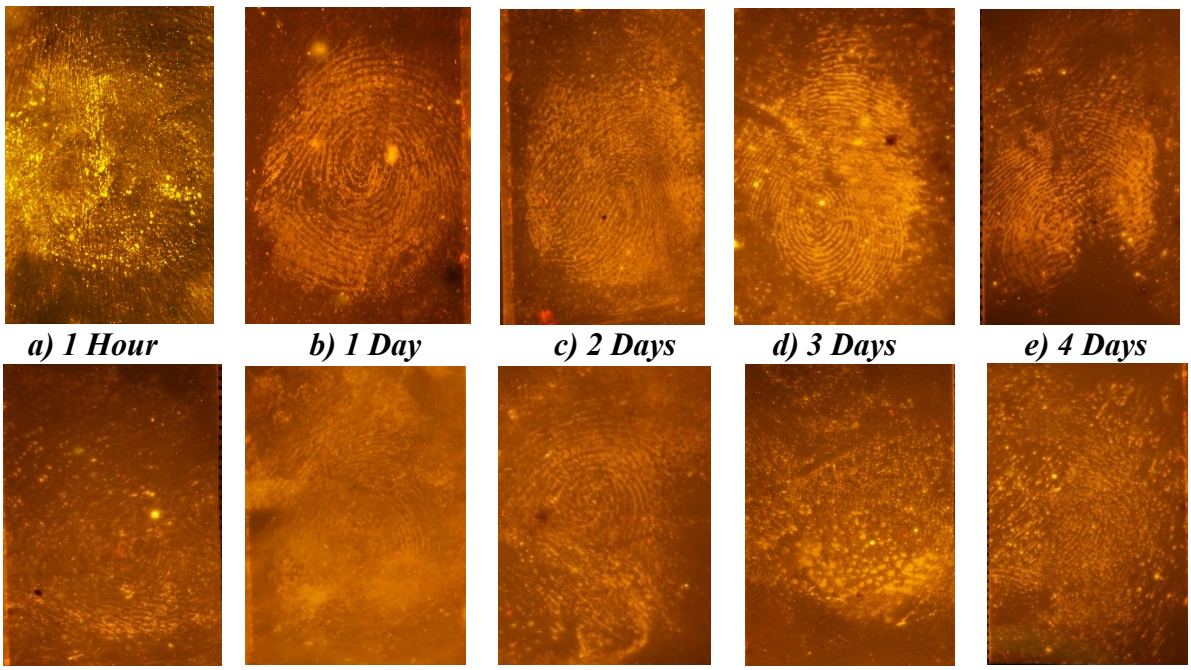
Figure 15: Hungarian Red Stained Blood Impressions Lifted with White Gellifters

Zar-Pro™ Fluorescent Lifters were able to recover and enhance blood impressions with visible proteinaceous materials and visible ridge details through most of the nine-day (B09) collection intervals. Some of the lifted impressions were visible under normal lighting conditions but most of the impression details were too faint to be visible without the use of alternate lighting (Figure 16a). The Lifters visualized under alternate lighting one hour after enhancement were not fluorescent as those visualized one month (Figure 16c) later, as the fluorescent properties of the Lifters are quenched by moisture. Thus, the Lifters need to be free of moisture to produce optimal fluorescence and all the featured photographs (Figure 17) were taken one month after lifting. A month timeframe allowed for more than adequate drying which optimized fluoresce within the proteinaceous impression affixed to the Lifters for improved visualization of impression details (Figures 17a-j). A month is not necessary to reach maximum fluorescence of the Lifters, but this timeframe was selected due to time constraints of the project. Zar-Pro™ Lifters are generally free of moisture within a few days after activation. This method allowed for the most reliable impression recovery with overall impression pattern and ridge details visible through most of the nine-day (B09) collection intervals (Figures 17a-j). One of the lifts was noticeably affected due to weather, the blood impression was covered in dew at the time of collection, causing impression to be smeared during the lifting process (Figure 17g). There was also some background fluorescence lifted from the pig skin as brighter contamination dots are visible on some of the lifted impressions (Figure 17b, d, e, and f). As decomposition progressed through the collection interval, impression details were lost, but throughout the daily progression fluorescent intensity remained bright (Figure 17a-j).



a) 1 Hour Post Lift *b) 1 Hour Post Lift* *c) 1 Month Post Lift*

Figure 16: Zar-Pro™ Lifted Blood Impressions



a) 1 Hour *b) 1 Day* *c) 2 Days* *d) 3 Days* *e) 4 Days*

f) 5 Days *g) 6 Days* *h) 7 Days* *i) 8 Days* *j) 9 Days*

Figure 17: Zar-Pro™ Lifted Blood Impressions

Non-impression controls were collected from the forehead (Figure 18) at one-hour (NC1), three-day (N03) and five-days (N05). The non-impression controls were used to assess contamination of lifted impressions due to artifacts associated with decomposition that may affect the visualization of impression details. As early as the one-hour (N01) collection interval there was visible contamination on the Zar-Pro™ Lifter, which was fluorescent when visualized under alternate lighting (Figure 19c) and contaminated the Lifters through the five-day (N05) collection interval. The white Gellifter collected visible proteinaceous materials (Figure 19a), and black Gellifter also had some fluorescent contamination (Figure 19b), both were visible starting at the 3-day (N03) collection interval. As there were no deposited proteinaceous materials in the non-impression control area, the presence of fluorescent materials in this area during the trials showed the preserved fetal pigs were secreting biofluids during the early stages of decomposition, most of which were fluorescent and contaminated the lifts when visualized under alternate lighting.

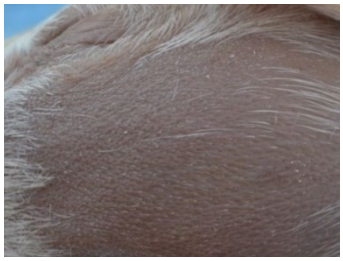
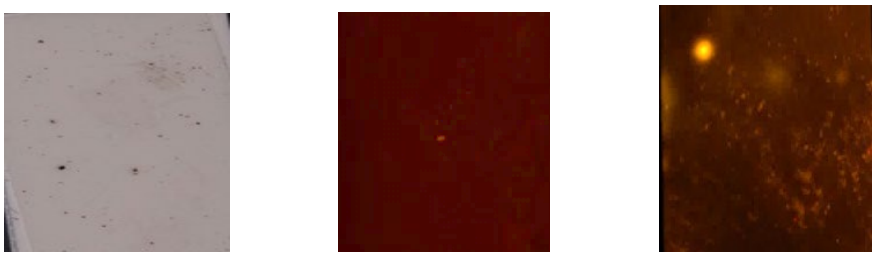


Figure 18: Non-Impression Control Area

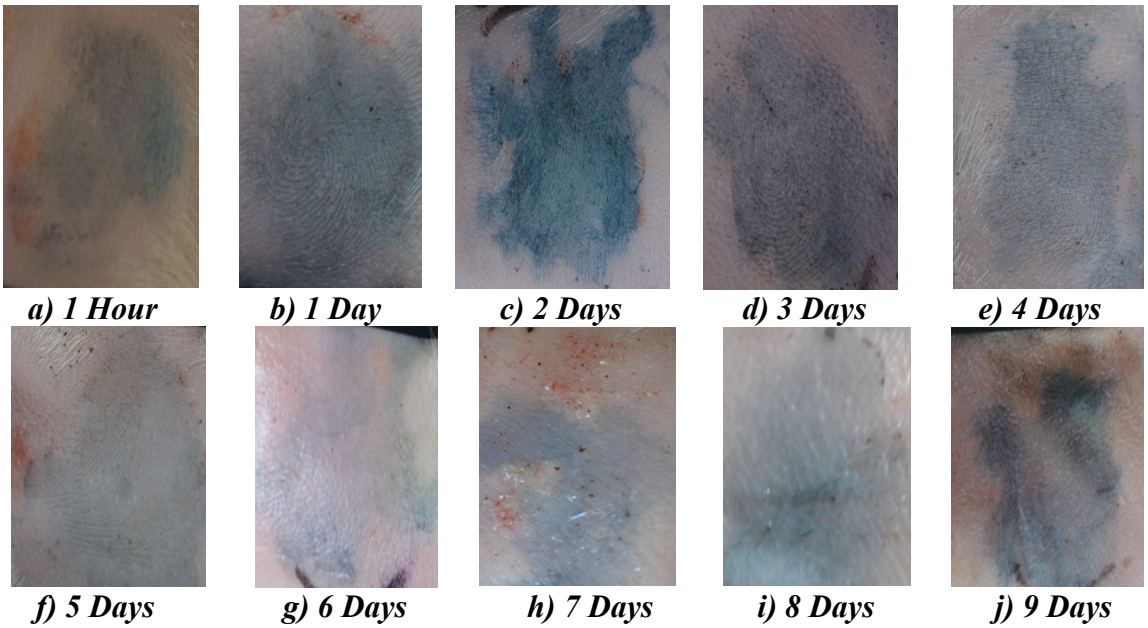


a) White Gellifters *b) Black Gellifters* *c) Zar-Pro™*

Figure 19: Non-Impression Controls

6. Results of Fresh Fetal Pig Trials- Fall 2021

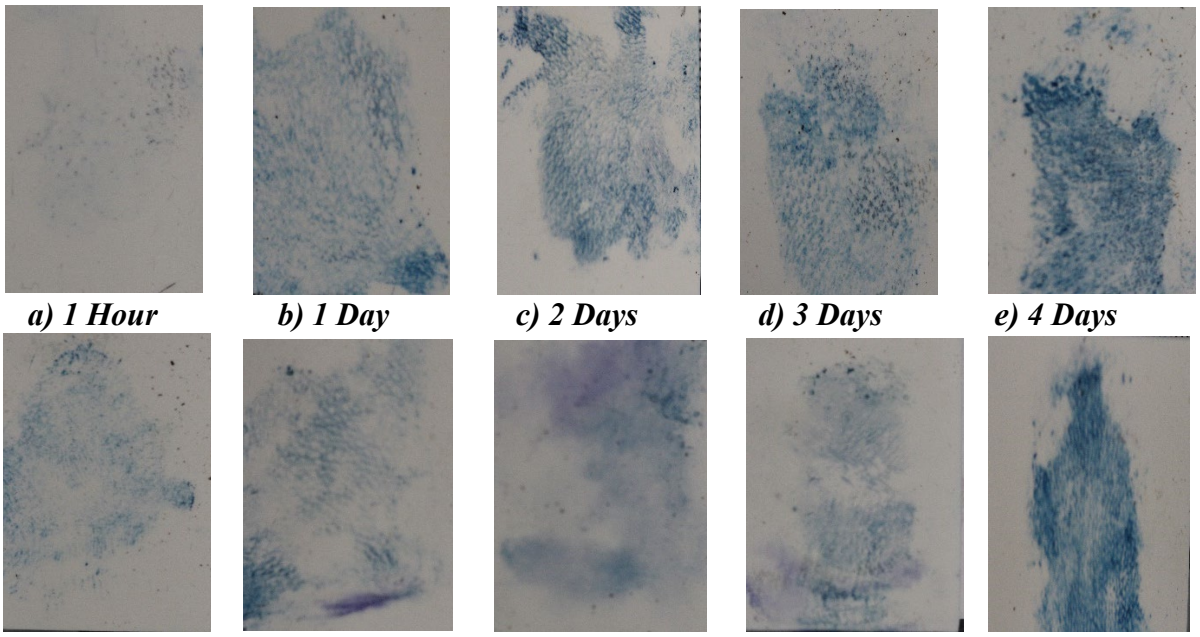
Amido Black dye-stained blood impressions were enhanced insitu on the fresh pig skin through the five-day (B05) collection interval (Figures 20a-e). After five-days the deposited blood impressions were affected by precipitation with the impression details being altered or destroyed from the moisture covering the impression area (Figures 20f-j). As with the preserved pig trials, the Amido Black stained impressions were not effectively lifted with the white Gellifters as the lifted impressions were smeared leading to the loss of impression details. All the featured photographs (Figure 21) of the lifted impressions were taken one hour after recovery as the impression details were more readily visible at this timeframe, and the stained impression details lightened when left on the Gellifters for a month.



a) 1 Hour *b) 1 Day* *c) 2 Days* *d) 3 Days* *e) 4 Days*

f) 5 Days *g) 6 Days* *h) 7 Days* *i) 8 Days* *j) 9 Days*

Figure 20: Insitu Amido Black Stained Blood Impressions

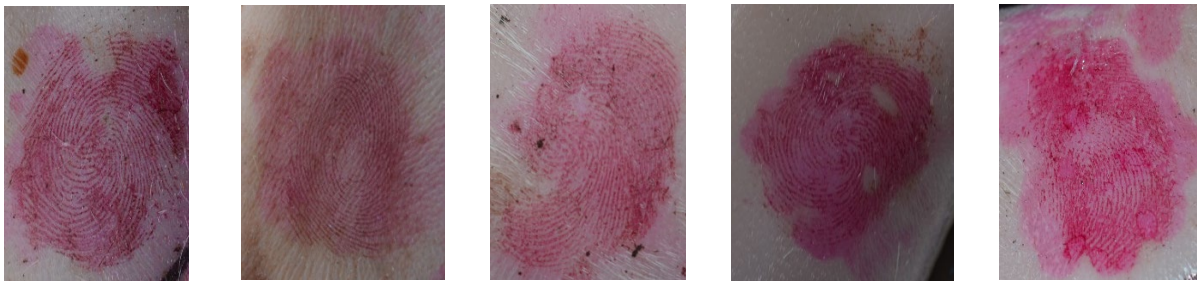


a) 1 Hour *b) 1 Day* *c) 2 Days* *d) 3 Days* *e) 4 Days*

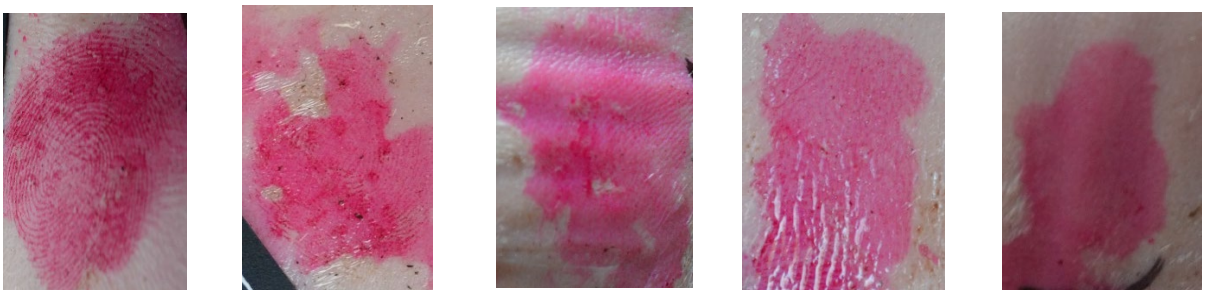
f) 5 Days *g) 6 Days* *h) 7 Days* *i) 8 Days* *j) 9 Days*

Figure 21: Amido Black Stained Blood Impressions Lifted with White Gellifters

Hungarian Red dye-stained impressions were more effective on the fresh skin than the Amido Black dye-stained impressions, allowing for impression details to be visible insitu on the pig skin through the six-day (B06) collection interval (Figures 22a-g) but were not as effective through the remaining nine-days(B09) (Figures 22 h-j). The Gellifters however, did not effectively lift impression details when visualized under normal (Figure 23a) or alternate lighting (Figures 23 b-j). All featured photographs (Figure 23) of the Gellifters were taken one hour after recovery, as impression details are lost when left on the lift for a month. The Hungarian Red dye stain was fluorescent on some of the Gellifters without visible impression details, however there was fluorescent fibers observed on the Gellifters when visualized under alternate lighting (Figures 23 f, i, and j), likely due to the cotton from the enhancement trays.

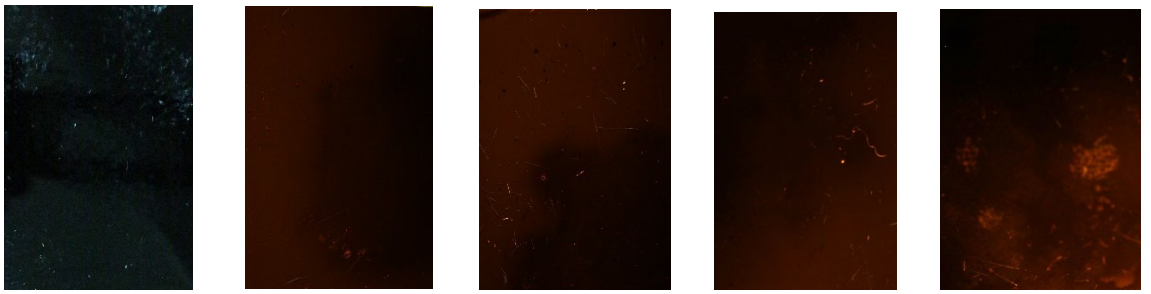


a) 1 Hour *b) 1 Day* *c) 2 Days* *d) 3 Days* *e) 4 Days*

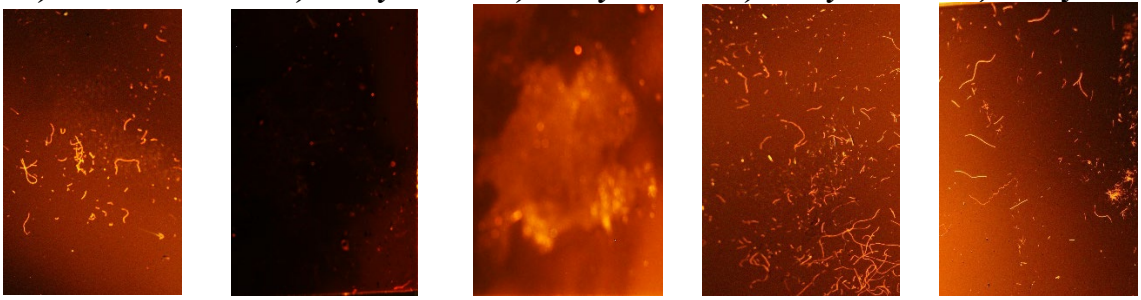


f) 5 Days *g) 6 Days* *h) 7 Days* *i) 8 Days* *j) 9 Days*

Figure 22: Insitu Hungarian Red Stained Blood Impressions



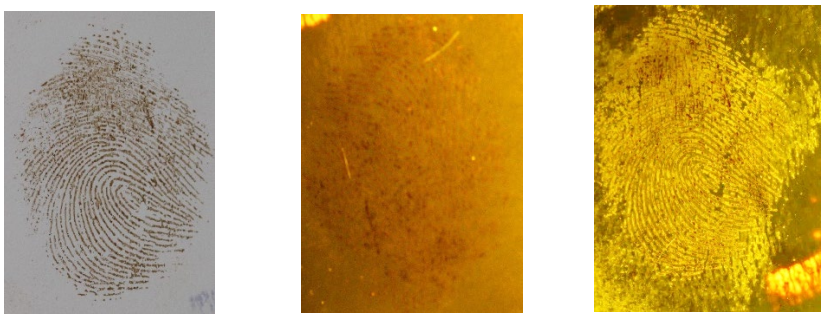
a) 1 Hour *b) 1 Day* *c) 2 Days* *d) 3 Days* *e) 4 Days*



f) 5 Days *g) 6 Days* *h) 7 Days* *i) 8 Days* *j) 9 Days*

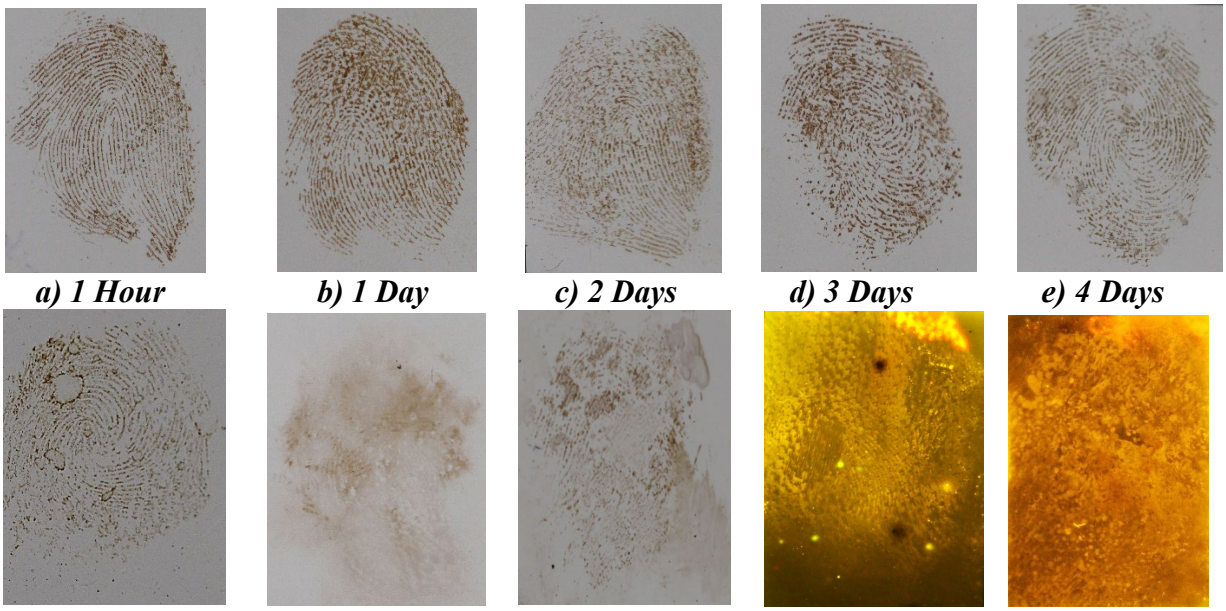
Figure 23: Hungarian Red Stained Blood Impressions Lifted with Black Gellifters

Zar-Pro™ Lifters effectively recovered blood impressions with visible impression details at the one-hour control interval (BC1) visualized under normal (Figure 24a) and alternate lighting (Figure 24b). But the fluorescent intensity of the proteinaceous impression on the Lifters visualized under alternate lighting one hour after recovery (Figure 24b) were not as brightly fluorescent as when visualized one month later (Figure 24c) as moisture in the Lifter from activation quenches fluoresce. Blood impressions were visible on the Lifters through most of the one-day (B01) through five-day collection intervals under normal lighting (Figure 25a-f). Blood impressions left insitu on the fresh pig skin were affected by precipitation resulting in the collection of smeared impressions when lifted from wet pig skin at the six-day (B06) (Figure 25g) and seven-day (B07) (Figure 25h) collection intervals when visualized under normal lighting. At the eight-day (B08) (Figure 25i), and nine-day (B09) (Figure25j) collection intervals only proteinaceous materials were visible even when visualized under alternate lighting. All featured (Figure 25) alternate lighting lifts were photographed one month after the collection to ensure moisture from the activation process was not quenching fluorescence.



a)1 Hour Post Lift *b) 1 Hour Post Lift* *c) 1 Month Post Lift*

Figure 24: Zar-Pro™ Lifted Blood Impressions

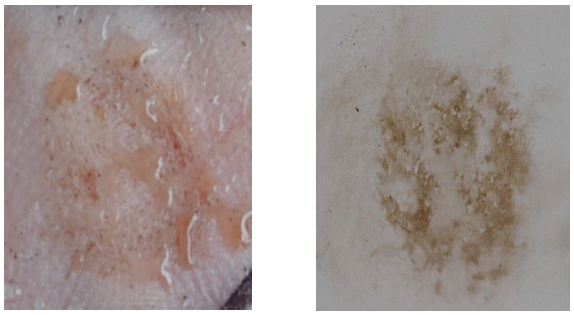


a) 1 Hour *b) 1 Day* *c) 2 Days* *d) 3 Days* *e) 4 Days*

f) 5 Days *g) 6 Days* *h) 7 Days* *i) 8 Days* *j) 9 Days*

Figure 25: Zar-Pro™ Lifted Blood Impressions

At the six-day interval, some of the impression areas had pooled water sitting on the pig skin prior to enhancement (Figure 26a) which as noted above, caused smearing, and smudging of the impression details when lifted with the Zar-Pro™ Fluorescent Lifters (Figure 26b) when visualized under normal lighting. The presence of precipitation was the most limiting factor associated with the effectiveness of the enhancement methods.



a) Wet Impression Insitu *b) Zar-Pro™*

Figure 26: Wet Impression Recovery

The non-impression controls collected from the forehead at the five-day (N05)(Figure 27) collection interval showed that there was no visible fluorescence observed under normal lighting on the white Gellifter (Figure 28a) nor on the black Gellifter (Figure 28b) under alternate lighting. Zar-Pro™ however did show some background fluorescence under alternate lighting (Figure 28c) indicating slight fluorescent contamination was coming from the fresh pig skin as a result of decomposition, but the contamination would not adversely affect the visualization of impression details.

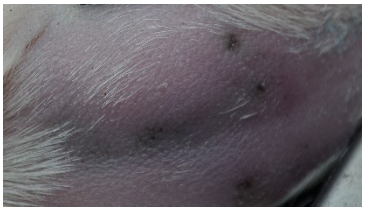
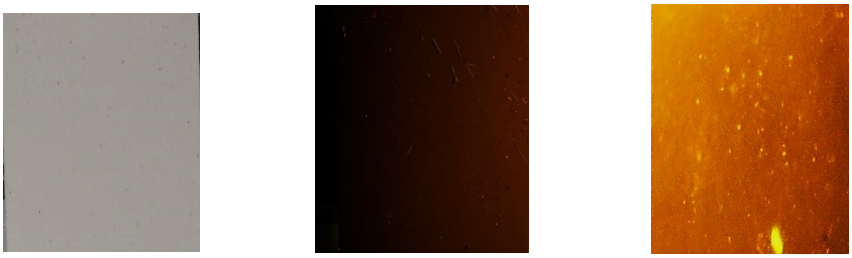


Figure 27: Non-Impression Control Area



a) White Gellifters ***b) Black Gellifters*** ***c) Zar-Pro™***

Figure 28: Non-Impression Controls

Significant Results

Amido Black and Hungarian Red dye-stained blood impressions were best when visualized in situ on the pig skin. The stained impressions, however, often lacked the desired contrast to visualize impression details, as the stain bonded to the blood but also absorbed into the semi-porous skin substrate creating background staining thus reducing visibility of impression details. Some of the Amido Black dye-stained impressions were able to be lifted from the skin using BVDA Gellifters® with partial impression patterns and some ridge path details visible on the white background of the lifter, but most of the lifted impressions had some proteinaceous materials with no ridge details visible. Hungarian Red dye-stained impressions lifted with BVDA Gellifters® were not effective at the recovery of impressions and were unable to lift any visible proteinaceous material from the skin onto the black background of the lifter under both normal and alternate lighting. Even with the fluorescent properties of Hungarian Red their overall effectiveness was limited, and the resultant fluorescence was weak. BVDA Gellifters® were not effective in lifting the dye-stained impressions and impression details were clearly lost in transfer. Zar-Pro™ Fluorescent Lifters were the only enhancement method that were able to lift the impression from the pig skin, with impressions having visible impression details when visualized both under normal and alternate lighting. The fluorescent properties of Zar-Pro™ allow for the visualization of impression details that were not readily visible under normal lighting.

7. Rating Assessment and Comparative Analysis of Preserved and Fresh Fetal Pig Skin

The compiled data set for the Preserved Fetal Pig trials contained 370 digital images for rating by the two student Examiners, whereas the Fresh Fetal Pig trials contained 403 digital images. The variation of images was due to the difficulty in recovering blood impression from the skin of the preserved pigs which was much more porous than the fresh pigs resulting in less recovered impressions throughout the collection intervals. The digital images were then labelled and archived into ten different data sets based on the daily collection interval throughout nine-days of trials and sent electronically to two student examiners for rating. The students rated both the Impression details (ID) and fluorescent intensity (FI) under both normal (NL) and alternate lighting (AL), and the resultant ratings were assessed using Cohens Kappa statistical analysis to measure inter- and intra- examiner variability.

The data sets and associated rating sheets for the fetal pig trials were organized, archived, and posted to Dropbox for rating by two student examiners which can be accessed via the following link:
https://www.dropbox.com/scl/fo/03sn4b8ienk4rjffu1kjz/h?rlkey=mu5td4cueu2nu6a2kykxwg822&dl=0

Based on the ratings between the two student examiners for the preserved pig trials, proteinaceous materials were visible on the pig skin prior to enhancement for most collection intervals through nine-days (Appendix C; Graphs 1, 5, and 11), with some days having an impression detail rating as high as a 3 (Appendix C; Graphs 1 and 11), but consistently between 1.5-2, meaning the impression patterns with ridge details were present prior to enhancement. The fresh pig trials had a slightly higher rating through the 5-day collection interval with several high impression detail ratings of 3 and the low rating of only 2 (Appendix C; Graphs 16, 20, and 26). After the five-day collection interval, the pre-enhancement ratings decreased to a high of 2 and a low rating of 0 through the nine-day interval (Appendix C; Graphs 16, 20, and 26), again meaning impression details were generally visible prior to enhancement.

The Amido Black dye-stained impressions visualized insitu on the pig skin were rated between 1.5 to a 3 for impression details through the six-day collection interval and then dropped to a low rating of 1 with a high of 2 through the remaining nine-days (Appendix C; Graph 2). The dye-stained impressions were lifted from the pig skin with white BVDA Gellifters® notably losing impression details with the one-hour ratings having only one impression detail rating of a 2 with several low ratings of 0 (Appendix C; Graph 3), meaning the visible ridge details from the dye-stained insitu impressions were not lifted with the Gellifters. The impressions affixed to the Gellifters had similar ratings when visualized one hour after recovery as they were one month later, however the impression details were faint, and the color was diffused making visualization more difficult (Appendix C; Graph 4). The fresh fetal pig trials produced similar results when compared to the preserved trials, with the dye-stained impressions visualized insitu on the pig skin rated between 0.5 to a 3 for impression details through the five-day collection interval and then dropped to a low rating of 0 with a high of 1 through the remaining nine-days (Appendix C; Graph 17). The white Gellifters were again not overly effective at lifting the dye-stained impression with a high of 2 and a low rating of 0.5 when rated one-hour after lifting (Appendix C; Graph 18), again meaning impression details were lost in transfer to the Gellifters. One-month after lifting, the impression detail ratings decreased again to a high of 1 (Appendix C; Graph 19), meaning only some proteinaceous materials could be visualized on the Gellifters.

The Hungarian Red dye-stained impressions visualized insitu on the pig skin were rated between 0.5 to a 2.5 for impression details through the nine-day collection interval (Appendix C; Graph 6). Black BVDA Gellifters® were then used to lift the dye-stained impressions which was less effective than the Amido Black stained impression with only three impression detail ratings above 0 with the high rating of only 1 when visualized one hour after recovery (Appendix C; Graph 7) and when visualized a month later all the ratings were at 0 when visualized under normal lighting (Appendix C; Graph 9). Due to the fluorescent properties of Hungarian red, impression details and fluorescent intensity were also rated under alternate lighting. One hour after recovery ratings under alternate lighting had a high impression detail rating of 1 with most of the ratings at 0 (Appendix C; Graph 8) and a month later the high rating was only 0.5 (Appendix C; Graph 10). The fluorescent intensity was rated a low of 3 and a high of 4 when visualized under alternate lighting one hour after recovery (Appendix C; Graph 8) and a month later the fluorescent intensity diminished to a high of 1.5 (Appendix C; Graph 10). Thus, the fluorogenic properties of the Hungarian Red dye stain did not assist in the visualization of impression details. Dye-stained impressions visualized insitu on the pig skin were rated between 0.5 to 2.5 for impression details through the nine-day collection interval. The fresh fetal pigs produced better results than the preserved fetal pig when using Hungarian Red to stain the blood impressions. The Hungarian Red dye-stained impressions visualized insitu on the pig skin were rated 3 for impression details through the five-day collection interval and then had a high of 1.5 with a low of 0 through the remaining nine-days (Appendix C; Graph 21). The black Gellifters were not effective at lifting the dye-stained impression with a high rating of 1 for impression details one hour after recovery (Appendix C; Graph 22) and a high rating of 0.5 one month later (Appendix C; Graph 24). The fluorogenic properties of Hungarian Red were not as fluorescent on the fresh pigs as they were on the preserved pigs with the high fluorescent intensity rating of 1 and a low rating of 0.5 one hour after recovery (Appendix C; Graph 23) and a month (Appendix C; Graph 25) later. As with the preserved trials, the fluorescent intensity did not improve the visualization of impression details with most impression details at a 0 under alternate lighting (Appendix C; Graphs 23 and 25).

The Zar-Pro™ Fluorescent Liters were not effective for the visualization of impression details under normal lighting from the preserved pig skin with a high rating of only 1 for impression details (Appendix C; Graph 12). The Lifters were much more effective on the fresh pig skin with impression detail ratings a high of 3 and a low of 1.5 through the five-day collection interval, which then decreased to a high of 1.5 and a low of 0 when visualized under normal lighting (Appendix C; Graph 27). The impression detail ratings increased when visualized under alternate lighting one hour after recovery with a high fluorescent intensity rating of 2, thus improving visualization of proteinaceous materials which was rated a 1 on the preserved pig skin (Appendix C; Graph 13). The ratings increased again when visualized under alternate lighting one month after recovery, as the Zar-Pro™ need time to dry, as moisture quenches fluorescence. One month after recovery the blood impressions lifted from the skin of the preserved pigs were still not visible under normal lighting (Appendix C; Graph 14) but when visualized under alternate lighting the impression details increased drastically to high ratings of 3 and low ratings of 1 and fluorescent intensity ranging from 0.5-4.5 (Appendix C; Graph 15). Thus, the fluorescent properties of the lifters allowed for visualization of impression details from the preserved pig skin through most of the nine-day collection intervals. The fresh pigs produced similar results to the preserved pigs, with impression detail ratings increasing slightly when visualized one hour after recovery under alternate lighting. The impression details had a high of 2.5 and a low of 0 and a fluorescent intensity rating ranging from 0-2.5 (Appendix C; Graph 28). One month after recovery impression details ratings were

similar to the rating from the one hour assessment under normal lighting (Appendix C; Graph 29). But due to moisture quenching fluorescence one hour after recovery the rating increased again with impression detail ratings a high of 3 and fluorescent intensity ranging from 2.5-5 through the five-day collection interval when visualize a month after recovery. Due to precipitation after the five-day interval for the fresh pigs, impression detail ratings decreased to a high of 1.5 with fluorescent intensity ranging from 1.5-2.5 (Appendix C; Graph 30).

Cohen's Kappa statistical analysis of the inter-and intra-examiner ratings were conducted for the two student examiners, identified as Examiner A and Examiner B for the preserved and fresh pig trials (Appendix D; Table 1). The intra-examiner assessment for Examiner A for the preserved pig trials was almost perfect for both impression details (0.959) and fluorescent intensity (0. 928) and was again almost perfect for the fresh pig trials for impression details (0.939) and fluorescent intensity (0. 984) but when re-rated the examiners rating for the preserved pig trials dropped to the lower end of almost perfect for fluorescent intensity (0.814) but remained similar for impression detail (0.981). The re-rating for the fresh pig trials remained almost perfect for impression details (1) and fluorescent intensity (0.965). In comparison the intra-examiner ratings for Examiner B were in almost perfect agreement for all ratings for the preserved pig trials with impression details (0.959) and fluorescent intensity (0.923) and the fresh pig trials impression details (0.992) and fluorescent intensity (0.993). The re-rated assessment still showed almost perfect agreement with the preserved pig trials impression details (1) and fluorescent intensity (0.950) and the fresh pig trials impression details (1) and fluorescent intensity (0.988).

Using Cohens Kappa statistical analysis, it was concluded that the preserved pig trials in Fall 2020 had more variability in rating assessments amongst examiners in comparison to the fresh pig trials from Fall 2021. The Cohen's Kappa value for the inter-examiner analysis of the fresh pigs had a fluorescence value of (0.986) and impression detail value of (0.997), which was a near perfect value according to the Cohens Kappa scale. However, for the preserved fetal pig skin, the fluorescence and impression detail values performed lower at (0.940) and (0.964), which supports the conclusion that the fresh pig skin was a better substrate than the preserved pig skin and should be selected as a proxy for human decedent skin for research purposes.

Significant Results:

Unlike in the preserved pig trials, the fresh pig skin behaved more like typical human skin. The preserved pig skin was more porous and spongier in comparison to the fresh skin, soaking up more of the dye stain than the fresh pig skin and even reducing the proteinaceous materials on the skin which affected visualization of the blood and limited bonding with the Gellifters and Zar-Pro™ Fluorescent Lifters. Through most of the nine-day collection intervals the blood impressions on the fresh and preserved fetal pig skin were notably visible, even if faint detection was not a significant concern. However, as expected detection of the blood on the preserved skin was more difficult than on fresh pig skin. Amido Black and Hungarian Red were effective enhancement methods for insitu dye-staining through the five-day collection intervals, with both stains having limited success through the nine-day collection intervals. The limited success in the fresh pig trials was in part due to precipitation which adversely affected enhancement after the five-day collection interval. Gellifters were not overly effective for the recovery of the dye-stained impressions in either the preserved or fresh pig trials. Even with the fluorescent properties of the Hungarian Red dye-stain, the fluorescent intensity was not significant enough to improve the visualization of impression details. It is also important to note that the fluorescent capabilities of Hungarian Red were diminished between visualization at one hour after recovery to month timeframe. The Zar-Pro™

Fluorescent Lifters were able to effectively bond to the proteinaceous materials in the blood impressions which were not always readily visible under normal lighting for the preserved pig trials, but impression details were visible under alternate lighting through most of the nine-day collection intervals. In the fresh pig trials, the Zar-Pro™ lifted impressions were visible under both normal and alternate lighting through most of the five-day collection intervals, however due to precipitation, impression details were lost through the remaining nine-days. Cohen's Kappa statistical assessment of examiner ratings determined almost perfect agreement between the two student examiners for both impression details and fluorescent intensity ratings. Thus, the examiners had significant agreement amongst the enhancement effectiveness for the recovery of blood impressions from the fetal pig skin. Fresh pigs had less variability than the preserved pigs indicating fresh pig skin is a better proxy to human skin and future studies should use fresh pig skin for research purposes.

8. Environmental Assessment for Fetal Pig Trials

A comparative analysis was conducted between six preserved and six fresh pigs which were used as a proxy for human skin to serve as a substrate for the enhancement of blood impressions collected over the course of nine-days in two subsequent years, 2020 and 2021. Weather patterns and environmental data were assessed to determine the effect of environmental factors on the recovery of blood impressions from pig skin during the early stages of decomposition. The pigs were placed in the same field under the same conditions in the fall season one year apart and were exposed to variant yet similar weather conditions. The efficiency of impression enhancement was compared to the daily weather, pig temperature, and rate of decomposition, as well as any other recorded pertinent information at each of the daily collection intervals.

Weather data was recorded at each collection interval, including temperature, humidity, precipitation, and dew point. The data was gathered from Weather Underground using the Detroit Metropolitan Airport as the nearest weather station, which is located 19 miles southeast of the MUFSRF, in Romulus, Michigan. Due to the nature of Michigan weather the trials which started only one day apart November 09, 2020, and November 08, 2021 had a range in temperature of 6.6°C. The preserved pig trials environmental temperature ranged from -1.1 to 25.5°C with the humidity varying 40-89%, and precipitation totals from 0-.69 inches with dew point ranging from 21.17 to 55.72°C (Table 1). The fresh pig trials also had a range of variability with the environmental temperature ranging from -2.8 to 18.9°C with the humidity varying 45-100%, and precipitation totals from 0-.33 inches with dew point ranging from 27.08 to 47.72 °C (Table 2). Thus, neither trial was free of precipitation and the weather conditions were not overly variant.

Interval	2020 Dates	Temp (C)	Humidity (%)	Precipitation (in)	Dew Point (F)
BC1	11/09	25.5	40	0	51.71
B01	11/10	17.2	63	0	55.72
B02	11/11	7.8	66	0.24	40.24
B03	11/12	3.9	73	0	29.71
B04	11/13	6.1	82	0	30.85
B05	11/14	0	81	0	26.79
B06	11/15	8.9	89	0.69	39.07
B07	11/16	3.9	65	0.14	25.56
B08	11/17	1.1	64	0.02	23.88
B09	11/18	-1.1	72	0.01	21.17

Table 1: 2020 Weather Data

Interval	2021 Dates	Temp (C)	Humidity (%)	Precipitation (in)	Dew Point (F)
BC1	11/08	18.9	45	0	40.33
B01	11/09	8.9	91	0	43.08
B02	11/10	0	100	0.01	37.34
B03	11/11	17.8	53	0	42.25
B04	11/12	7.8	50	0.33	32.31
B05	11/13	5.6	72	0	31.24
B06	11/14	1.1	89	0.01	30.89
B07	11/15	1.1	85	0.19	28.38
B08	11/16	-2.8	95	0	27.08
B09	11/17	11.1	90	0	47.72

Table 2: 2021 Weather Data

The temperature of the pig skin was measured at each collection interval using a digital thermometer. Over the course of the collection intervals in both trials the pig skin was variant from the one-hour collections (BC1) through the nine-day interval (B09) (Tables 3 and 4). The preserved pig skin temperatures started at an average of 16.6°C, whereas the fresh pig skin averaged 15.03°C. During both trials the pig temperature dropped over the following days with the preserved pigs dropping in temperature through the five-day interval (B05), averaging -13.1°C, before warming again until the last collection intervals at days eight (B08) and nine (B09), averaging -13.9°C. The fresh pig temperatures dropped over the two-day collection interval (B02) to an average 4.3°C, before rising back up near the temperatures from the one-hour interval (BC1). The pig temperatures then dropped again through the eight-day collection interval (B08), averaging -1.35°C, before rising again for the last collection interval at day nine (B09), averaging 8.7°C.

2020 Trials Preserved Pigs Interval	BC1	B01	B02	B03	B04	B05	B06	B07	B08	B09
Date	11/09	11/10	11/11	11/12	11/13	11/14	11/15	11/16	11/17	11/18
Pig 1	19.9 °C	14.7 °C	0.6 °C	-2.3 °C	-2.9 °C	-13.9 °C	3.3 °C	-1.8 °C	-10 °C	-12.5 °C
Pig 2	18.7 °C	12.8 °C	0 °C	-3.9 °C	-3.2 °C	-12.8 °C	4.6 °C	-5 °C	-13 °C	-12.6 °C
Pig 3	20.6 °C	13.6 °C	0.6 °C	-3.5 °C	-3.4 °C	-12.8 °C	4.2 °C	-5.6 °C	-13.5 °C	-13 °C
Pig 4	20.2 °C	14.2 °C	0.6 °C	-4.8 °C	-3.9 °C	-12.8 °C	3.3 °C	-3.3 °C	-14.2 °C	-12.1 °C
Pig 5	20.5 °C	14.5 °C	0 °C	-3.6 °C	-4 °C	-12.8 °C	3.8 °C	-5.6 °C	-17 °C	-15.1 °C
Pig 6	20 °C	14.4 °C	0 °C	-4.9 °C	-3.8 °C	-13.3 °C	3.9 °C	-2.2 °C	-18.7 °C	-14.9 °C

Table 3: Daily Pig Skin Temperatures- Fresh Pig Trials

2021 Trials Fresh Pigs Interval	BC1	B01	B02	B03	B04	B05	B06	B07	B08	B09
Date	11/08	11/09	11/10	11/11	11/12	11/13	11/14	11/15	11/16	11/17
Pig 1	14.5 °C	6.3 °C	3.8 °C	14.2 °C	9.7 °C	6.1 °C	1.9 °C	0.5 °C	-2.3 °C	9.9 °C
Pig 2	14.4 °C	5.8 °C	4.2 °C	13.6 °C	9.2 °C	5.9 °C	3.5 °C	1.4 °C	-1.5 °C	8.8 °C
Pig 3	15 °C	6 °C	4.3 °C	14.3 °C	9.1 °C	6.1 °C	2.1 °C	1.1 °C	-0.9 °C	8.4 °C
Pig 4	15.2 °C	5.5 °C	4.8 °C	15 °C	9.1 °C	6.2 °C	3 °C	0.9 °C	-1.2 °C	8.5 °C
Pig 5	15.9 °C	6 °C	4.5 °C	15.8 °C	9.2 °C	5.3 °C	3.1 °C	1.5 °C	-1.4 °C	8.3 °C
Pig 6	15.2 °C	6.1 °C	4.2 °C	14.6 °C	8.8 °C	5.9 °C	2.3 °C	1.4 °C	-0.8 °C	8.5 °C

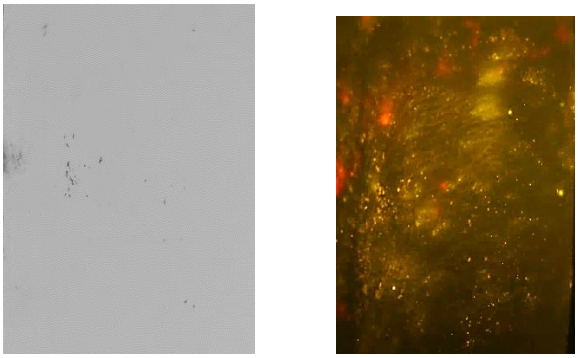
Table 4: Daily Pig Skin Temperatures- Preserved Pig Trials

Weather conditions, along with the temperature of the pigs and insect activity did affect impression enhancement throughout both the preserved and fresh pig trials. The six pigs for each of the trials appeared to decompose at the same rate, in accordance with the respective intervals in each trial. A lot of the same observations were made for each pig, with some differences regarding the coloration and condition of the eyes, and the rate at which the skin began to decompose. Fly activity around the pigs was noted as soon as the pigs were placed in the field area for decomposition. As decomposition progressed the flies were focused around orifices on the pigs such as the umbilical cord, eyes mouth and ears. As early as the one-day interval (B01), the pigs had fluid seepage with early signs of skin discoloration. During the preserved pig trials an overnight rainfall (Table) before the two-day collection intervals (B02) left accumulated moisture on the pigs but pigs 2 and 5 specifically which had pooled water on top the impression areas affecting impression recovery. The fresh pigs were not affected by moisture and were continuing the beginning stages of decomposition with more noted skin discolorations. The preserved pigs were again affected by moisture at the three-day collection interval (B03) as the temperatures had dropped overnight and the pigs were damp from frost. The preserved pig skin was now turning more of a greyish color and becoming dehydrated, which was seen by the curling of the pig tongues. At this collection interval for the fresh pigs, fluid was accumulating on the trays with fluid leaking from the orifices, such as the nose and mouth. Flies were now laying eggs in the open orifice and the eyes were dehydrating.

Moisture continued to be present on the pig skin during the preserved trials and was beginning to accumulate on the pig skin by the five (B05) and six-day collection interval (B06) for the fresh pigs. The pigs were continuing to decompose with areas of the pigs being de-fleshed and the skin was starting to turn a dark color with injured areas beginning to appear. For both preserved and fresh pigs, the six-day collection intervals had a lot of accumulated moisture on the pigs. The preserved pigs had pooled water on impression areas, and the fresh pigs had been covered with snow, altering the recovery of impression details for both trials. At this interval, the pigs were now a darker colored pink with some greenish-black skin discoloration due to decomposition. At the seven-day collection interval (B07) the preserved pigs were still progressing in decomposition but not at the same rate that the fresh pigs progressed. The fresh pigs were covered in a thin layer of moisture that had formed ice in various areas on the skin. Most of the pigs were discolored now with the pig legs blackened and the skin now being de-fleshed in multiple areas on the pigs. For the eight-day collection intervals (B08), both pig trials were affected by snowfall. The preserved pigs had a light cover of snow on the skin and the fresh pig trial were covered in snow with the liquid accumulated from decomposition frozen on the trays. Both pig trials continued to decompose with

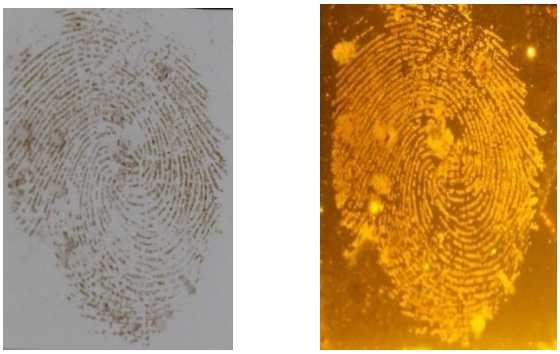
more drastic coloration in the fresh pigs and insect activity was no longer readily noted. At the nine-day collection intervals (B09) the preserved pig skin was a greyish color with areas of darker green-black coloration but did not appear to be decomposing any further than this stage. Whereas the fresh pigs were still progressing in decomposition. A browner color liquid in addition to the reddish colored seepage was now present on the pig skin and accumulated in the trays. The abdominal areas were swollen, and skin discoloration and de-fleshing of some areas continued. At this last collection interval for both pigs, the skin was still intact, thus impression could potentially be recovered longer. Leaf litter was frequently found on the pigs throughout both trials but did not affect impression recovery.

The environmental factor that had the biggest impact on impression recovery was precipitation. During the fresh and preserved trials, the pigs were exposed to consistent moisture with high humidity and precipitation. The preserved pigs were affected by moisture through most of the collection intervals, especially in the last days (Figure 29a and b). The amount and duration of snowfall left the pig skin wet and some of the impressions were washed away, thus making impression recovery more difficult. The fresh pigs were also affected by moisture and snowfall throughout the trials (Figure 30a and b), with the two-day collection interval recording 90% humidity with a dew point of 47.72in. The cold weather throughout the collection intervals did not appear to affect impression recovery, but precipitation had a major effect on impression recovery, regardless of enhancement method. Water from rain or snowfall when present washed away the impression, thus no enhancement of the affected areas was overly effective.



a) Normal Lighting b) Alternate Lighting

Figure 29: Preserved Pig Trials Zar-Pro™ Lifter destroyed by Snowfall



a) Normal Lighting b) Alternate Lighting

Figure 30: Fresh Pig Trials Zar-Pro™ Lifter with Precipitation Drops

Significant Results

The condition of the pigs and impressions were compared to the data sets to examine correlations between the weather conditions and the quality of impressions recovery at each collection interval during the early stages of decomposition. Throughout the duration of the trials, the preserved and fresh fetal pigs did not have any substantial insect activity and the skin stayed intact for the nine-day duration of the trials, thus future trials could continue past nine-day collections for fetal pigs. The preservatio9n of the pig skin through nine-days may have been in part due to the colder weather during the seasonal fall trials. The environmental factor that had the most significant impact on the ability to enhance and recover the blood impressions from fetal pig skin was precipitation. The preserved pigs were exposed to rainfall prior to the two-day collection interval and then again at the three-day interval however it wasn't until the six-day collection interval that the preserved pigs were significantly affected with moisture accumulating on the skin. The fresh pig trials were also affected by precipitation at the five and six-day collection intervals with moisture accumulating on the skin. Moisture on the skin adversely affected the ability of the dye-stains to bond to the proteinaceous impressions and created smearing and smudging on the Zar-Pro™ Lifters due to the moisture smearing the blood during the lifting process. Thus, precipitation in the form of rain or snowfall was concluded to be the environmental condition that affected the ratings most during both trials. The ratings of the impression details decreased as soon as precipitation was noted with accumulated moisture on the skin, around the five-day collection intervals for both the preserved and fresh pig trials.

IV. Human Decedent Trials

1. Anthropological Research Facilities

The Northern Michigan University (NMU) facilities and research and educational programs were instituted in 2017, and the first human donors were laid out at FROST during the summer of 2018. The program has averaged approximately six donors per year, and as of December 2022 received a total of 34 donors. Twenty-nine of our donors have been next-of-kin donations, meaning after their deaths, their legal next-of-kin has chosen to donate their remains to our program. Five of our donors have been self-donors, meaning they signed paperwork prior to their deaths and expressed their desire to donate to NMU to their next-of-kin, who then honored their wishes at the time of their deaths. The NMU Center for Forensic Anthropology (CFA) donors consist of 11 females and 23 males; their ages-at-death range from 37 years to 92 years, with an average age-at-death of 73 years. One donor self-reported as identifying as Native American, while the other donors all identified as "White or Caucasian." Two donors were unpulverized cremains; the other 32 have spent some time (ranging from weeks to years) in the outdoor facility (FROST) before being transferred to the indoor facility (FARL) to be cleaned, analyzed, labeled, and curated.

The FROST facility is approximately one acre in area, surrounded by an eight-foot chain link fence that is capped with three rows of razor wire. The land on which the facility sits was formerly owned by the Marquette Branch Prison and was legally transferred by the Michigan Legislature to NMU in 2017. The eastern, northern, and western perimeters of the property are surrounded by prison property; the southern perimeter borders the parking area of another state-level agency. To ensure privacy for the human donors and integrity of the research, plastic privacy slats are woven through the chain link, and an opaque black fabric is attached to the internal surface of all fence lines. It is not possible to see the inner area of the field from the outside of the fence. Two security cameras monitor the entrance to the site and one security camera monitors the south fence line. The

NMU Police Department monitors all cameras 24/7. The facility is equipped with two swing gates, which allow for passage of vehicles, such as funeral home delivery vehicles and lawn maintenance vehicles, into and out of the fenced area. A small, enclosed gravel-paved area inside the fence allows for limited parking and staging of supplies. There is also a small, insulated on-site building, which provides space for storage of equipment, samples, and personal protective equipment, office supplies, and field gear.

The FARL facility is an approximately 3,000 sq ft. space, which was renovated in 2017-2018. The building consists of two offices, a conference room, a mechanical room, two bathrooms, an analysis laboratory, a processing laboratory, and a large garage space. The analysis laboratory contains five analysis tables, which are dedicated to laying out human skeletal remains. This laboratory is also equipped with a dissecting microscope, bookshelves, photography equipment, and shelving for the curated donor skeletal collection. The processing laboratory is equipped with stainless steel countertops and upper and lower cabinets, a restaurant-style sink, a chemical fume hood, and an 80-gallon steam kettle for macerating human remains. This laboratory is also a controlled-access space where forensic cases can be secured. The garage space allows for storage of a CFA vehicle and archaeology equipment, and houses four large mortuary-style cooling units, two three-body freezers and two three-body refrigerators.

2. Decedent (Donors)

All human donated remains are given a unique numerical identifier, consisting of a two-digit year of intake, followed by a dash, and a three-digit number indicating the sequence in which they are received (e.g., 18-001, 18-002, etc.). For the purpose of maintaining donor confidentiality, no donor numbers are utilized in this project. The project-specific donors are referred to only as "Donor 1" or "D1," "Donor 2" or "D2," and "Donor 3" or "D3." This sequence of numbers is related to the order in which fingerprints were deposited and the donors were placed in the outdoor facility and is unrelated to both their actual donor numbers and the order in which they were received at the NMU CFA facility.

The three human donors utilized for this project arrived at the FARL facility in November (n = 2) and December (n = 1) of 2020 and were stored in one of the mortuary freezer units until July 9, 2021, at which time they were transferred to one of the refrigeration units, where they remained until July 20, 2021. While in the freezer units, the donors were maintained at approximately -4ºC. The freezers occasionally undergo a defrost cycle, and the temperatures range from -9.5ºC to 2ºC, so the -4ºC is both the freezer's set temperature and the overall average. While in the refrigeration units, donors were maintained at an average temperature of 4ºC. On July 20, 2021, Madonna University researchers applied blood impressions and semen smears onto the decedent donors' skin and collected the one-hour (BC1 and NC1) controls while in the FARL processing lab. The donors were then transferred to the FROST facility for the duration of the research project.

Donor 1 (D1)

Donor 1 was an 82-year-old White male, approximately 5'10" in height and weighing approximately 190lbs at the time of his death. Donor 1 was an alcoholic of many years and eventually died due to complications related to cirrhosis of the liver. He arrived at the FARL in December of 2020, on the day of his death. His transfer of custody paperwork was completed, then he was photographed and placed in a mortuary freezer, where his body remained until July 9, 2021. On July 9, 2021, Donor 1 was transferred to a mortuary cooler. The condition of his body was checked on July 18, 2021, at which time the decision was made to keep him in the cooler until July

20, 2021. On that date, at approximately 8:00am, the team from Madonna University, assisted by two NMU students, transferred the donor to our processing laboratory to deposit fingerprints and semen smears relevant to this research. Donor 1 was placed in the outdoor facility at approximately 2:00pm on the same day, following impression deposition and the collection of the one-hour (BC1, SC1, and NC1) controls.

Donor 2 (D2)

Donor 2 was a 70-year-old White female. She was approximately 5'5" tall and weighed nearly 200lbs at the time of her death. Donor 2 had suffered from hypertensive cardiovascular disease for a number of years prior to her death, and eventually died of heart failure. She arrived at the FARL in December of 2020, one day following her death. Her transfer of custody paperwork was completed, then she was photographed and placed in a mortuary freezer, where her body remained until July 9, 2021. On July 9, 2021, Donor 2 was transferred to a mortuary cooler. The condition of her body was checked on July 18, 2021, at which time the decision was made to keep her in the cooler until July 20, 2021. On that date, at approximately 8:00am, the team from Madonna University, assisted by two NMU students, transferred the donor to our processing laboratory to deposit fingerprints and semen smears relevant to this research. Donor 2 was placed in the outdoor facility at approximately 2:00pm on the same day, following impression deposition and the collection of the one-hour (BC1, SC1, and NC1) controls.

Donor 3 (D3)

Donor 3 was a 38-year-old White male who had experienced an anoxic brain injury approximately two years prior to his death, which left him a quadriplegic for his remaining years. His death was due to respiratory failure, a complication of bacterial pneumonia. He arrived at the FARL in December of 2020, three days following his death. His transfer of custody paperwork was completed, then he was photographed and placed in a mortuary freezer, where his body remained until July 9, 2021. On July 9, 2021, Donor 3 was transferred to a mortuary cooler. The condition of his body was checked on July 18, 2021. This donor was very thin at the time of his death, meaning he thawed more quickly than the others. When his condition was checked on July 18, 2021, he had begun to show signs of advancing decomposition. At that time, he was returned to the freezer. Placement back into the freezer was intended to slow the decomposition process to ensure that there would be three research subjects dedicated to this study. On July 20, 2021, Donor 3 was returned to the cooler. On July 21, 2021, at approximately 9:00am, the team from Madonna University, assisted by two NMU students, transferred the donor to our processing laboratory to deposit the fingerprints relevant to this research. Donor 3 was placed in the outdoor facility at approximately 2:00pm on the same day, following impression deposition and the collection of the one-hour (BC1 and NC1) controls.

3. Human Subjects/Study Population

This research is exempt from Institutional Review Board (IRB) research as, the human subjects are deceased thus they do not qualify as human subjects and the donors themselves, are not the focus of this research. Madonna University Institutional Review Board has reviewed the research and provided exemption status from Federal Policy for the Protection of Human Subjects of the United States Government. The FROST facilities employ site-specific, proprietary tracking systems for their donors, which are and will remain unknown to all other participants in this study. All Co-PI's and assistants associated with this project will have access only to the unique donor numbers,

which will be separated from any personally identifying information (PII) or personal health information (PHI) prior to their placement in the outdoor facility. To further ensure donor confidentiality, all photographs of the human donors associated with this project focused only on the study regions and were labeled according to the study-specific numbering system, and do not include visually identifying information.

4. Decedent (Donor) Variability

This study involved the use of human decedent donors, specifically the skin of the deceased, as the skin was to serve as a substrate for blood impression and semen smears in order to test the ability to enhance and recover impressions which is the actual focus of the research. Variables specific to each donor such as time of death, cause of death, skin tone, age, weight, condition of the skin and hair, and any associated patterns and textures were recorded for each of the three decedent donors. Due to the condition of Decedent 3 (D3), specifically his ante- and postmortem condition, he was deemed noncomparable to Decedents 1 (D1) and 2 (D2) for research purposes. In addition to D3's advanced state of decomposition, the contorted state of his body, and the condition of his skin did not allow for the number of required deposited blood impressions necessary to complete the data set for the duration of the research trials. Therefore, only D1 and D2 were utilized in the primary research trials and D3 had a smaller data set that was used to augment the research.

5. Deposition of Blood Impressions and Semen Smears

Three student research assistants deposited optimal quality blood impressions onto the skin of the decedent donors for the trials. Depositors rotated equally between the decedent donors, so no single research assistant was depositing all their impressions onto one donor or impression area to ensure for comparable variation amongst impressions. Each deposited impression was then assessed to ensure optimal quality (ID rating 3), meaning visible proteinaceous material, visible ridge detail to include the overall impression pattern, ridge paths and deviations. The deposition of optimal quality impressions ensures that changes to the impression during the trials are a result of degradation during the early stages of decomposition and not due to variation in deposition. Semen could not be optimized for the deposition of an optimal quality impression thus semen smears were deposited on the decedent donors for the trials.

The original research plan was to deposit 145 blood impressions and semen smears onto the skin of all three decedents, totaling 435-deposited impressions. After assessing the decedent donors prior to the start of the trials, it was determined Decedent 3 was not comparable to Decedents 1 and 2 as this donor had open wounds and had already advanced into the beginning stages of decomposition. Additionally, the availability of accessible skin on Decedents 1 (D1) and 2 (D2) was limited thus only 130 blood impressions were deposited onto these two decedents at the FARL. A total of 24 blood impressions were deposited on D1 and D2 in the following pre-determined body locations: right arm (LRA), left arm (LLA), right leg (LRL), left leg (LLL), and neck area (LNK) and another 10 impressions were deposited on the chest area (LUC). Note: the neck area (LNK) included the neck and upper chest, whereas the upper chest area (LUC) was more the lower chest just above the rib cage. The forehead (LFH) was impression free and designated as a non-impression control (NC) area for each decedent. An additional 21 semen smears were placed on the inner thigh (LIT) area of D1 and D2. Note: the inner thigh area was more of the upper pelvic area. Due to the condition of D3, only 33 blood impressions were deposited on the decedents skin with 9 impressions placed on the right arm (LRA) and left arm (LLA), and 15 impressions placed on the upper left leg (LLL). In total, 293 blood impressions and 42 semen smears were deposited between the three decedent

donors. The deposited blood impressions and semen smears were marked with a half-circle using a black sharpie to ensure that if the impressions became latent the impression area could still be targeted for enhancement.

The impression map (Appendix B; Figure 1) was developed to serve as a guide for the deposition of blood impressions, semen smears and non-impression controls as well as providing an identifying code indicating decedent donor, impression location, collection interval, and enhancement method. Decedent donors were identified as D1, D2, and D3 with the impression locations of neck area (LNK), left arm (LRA), right arm (LLA), upper chest (LUC), left leg (LLL), right leg (LRL), inner thighs (LIT) and the non-impression control location of forehead (LFH). Collection intervals for blood impressions ranged from the blood control (BC1) collected one-hour after deposition and then daily in the field at the one-day (B01) through ten-day (B10) collection intervals. Collection intervals for semen smears ranged from semen control (SC1) collected one-hour after deposition with a daily collection from one-day (S01) through five-days (S05). Non-impression controls (NC1) were collected at one-hour, three-day (N03) and five-day (N05) collection intervals. The three-enhancement methods: Amido Black lifted with a white BVDA Gellifters® (EA), Hungarian Red lifted with a black BVDA Gellifters® (EB), and Zar-Pro™ Fluorescent Lifters (EC) were used to enhance impressions at the various intervals during early stages of decomposition. Non-impression controls were collected using Zar-Pro™ Fluorescent Lifters (ZP) and BVDA Gellifters® (GL) to assess the effect of any inherent fluorescent properties associated with decomposition on the visualization of the enhanced impressions.

6. Field Placement

The one-hour blood control impressions (BC1), one-hour semen control smears (SC1) as well as one-hour non-impression controls (NC1) were collected at the FARL facility prior to the transportation of the decedents (D1, D2, and D3) to the FROST facility. After collecting the controls in the refrigerated laboratory at FARL, the decedent donors were carefully transported to the FROST facility where they remained for the duration of the trial. For transport, the decedent donors were packaged in a cardboard box with plastic sheeting, the boxes were left open on the top and covered with a plastic tarp which were then placed on a wooden transportation tray (Figure 31). The box was left open to prevent the destruction of the blood impressions and semen smears during transportation via the CFA vehicle and final placement at FROST. The decedents were placed in pre-determined sites (Global Positioning Satellite (GPS) recorded) in a North South Orientation on the FROST facility grounds. The decedents were positioned in their field placement using the bottom piece of the cardboard and plastic cloth to allow for the movement of the decedents from the gurney without touching the bodies as to preserve the blood impressions and semen smears that were deposited onto the decedent skin. They were positioned on their back (supine position) and remained unclothed for the duration of the research trials (Figures 32a-c).

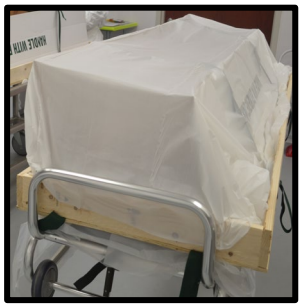


Figure 31: Transportation of Decedent Donors

a) Donor 1 *b) Donor 2* *c) Donor 3*

Figure 32: Field Placement of Decedent Donors

7. Collection Intervals and Storage

The remaining blood impressions were collected outdoors at the FROST facility from D1 and D2 at: one-day (B01), two-day (B02), three-day (B03), four-day (B04), five-day (B05), six-day (B06), seven-day (B07), and eight-day (B08) collection intervals. The additional blood impressions were recovered from D1 through eight days (B08) and from D2 through the ten-day collection interval (B10) both of which were enhanced with the Zar-Pro™ Fluorescent Lifters (EC). Semen smears were also collected from D1 and D2 at: one-day (S01), two-day (S02), three-day (S03), and five-day (S05) collection intervals. Non-impression controls were collected from D1 and D2 at: one-day (N01), two-day (N02), three-day (N03), five-day (N05), and seven-day (N07) collection intervals. Impressions were only recovered from D3 at the one-hour (BC1), one-day (B01), two-day (B02), three-day (B03), four-day (B04), and five-day (B05) collection intervals with non-impression controls collected at the one-day (N01), two-day (N02), and three-day (N03) collection intervals. After each trial, a protective cage was placed over the decedents to protect against large scavengers and a canopy was placed over the caged areas to buffer against direct rainfall. The canopy also served to protect researchers and their equipment from inclement weather.

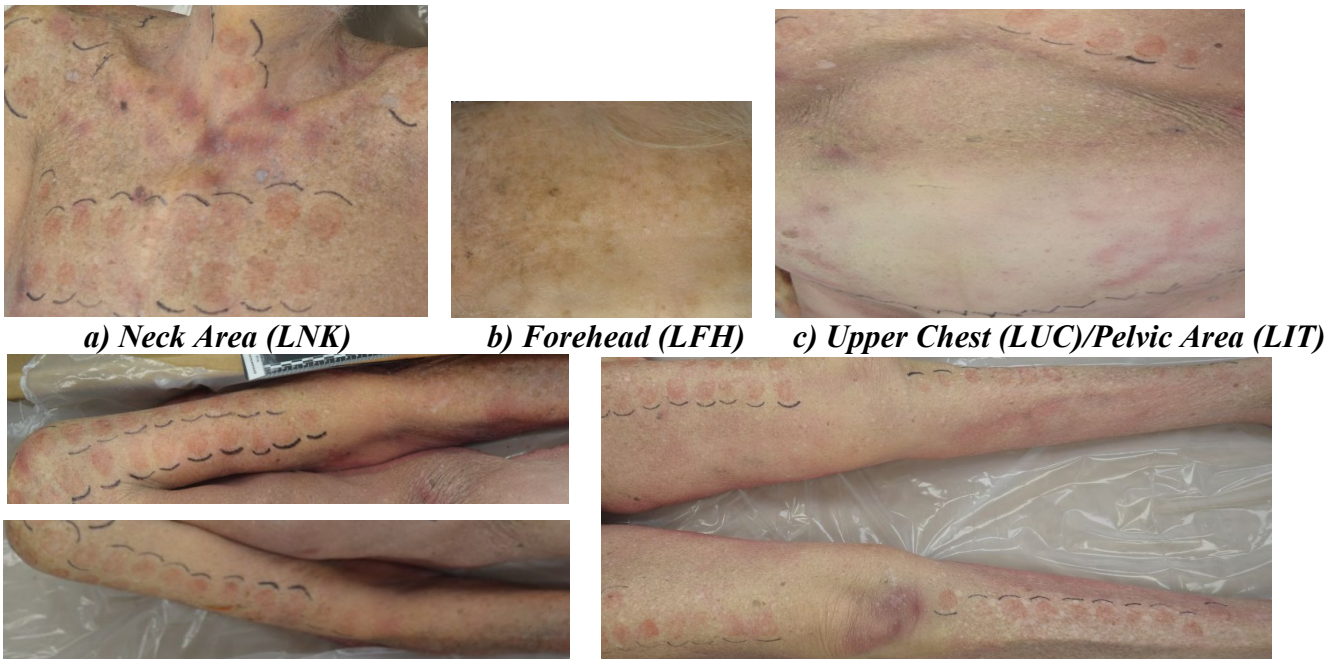
A total of 260 blood impressions were deposited on the skin of D1 and D2 with 237 being recovered (23 were not recoverable) over ten days of decomposition using Amido Black dye-stain lifted with white BVDA Gellifters® (EA), Hungarian Red dye-stain lifted with black BVDA Gellifters® (EB), and Zar-Pro™ Lifters (EC). Semen smears were also deposited on the skin of D1 and D2 with 30 smears deposited and 24 of them recovered through the five days of decomposition. In addition to the recovered blood impressions and semen smears another 54 non-impression controls were lifted using white and black BVDA Gellifters® (GL) and Zar-Pro™ Lifters (ZP). All lifted impressions and non-impression controls were stored individually with a unique identifier in pre-labelled dye-free white envelopes and then grouped by collection interval in larger pre-labelled manila envelopes and stored in plastic filing totes. The compiled digital images collected via photography at each collection interval were also labelled and archived with duplicate copies saved to a secure, password protected external hard drive. Recovered lifts, non-impression controls and archived photographs were transported to MUFSRF for subsequent analyses. An inventory chain of custody log was maintained to ensure sample integrity. All physical samples are being stored in a locked file cabinet in a controlled access storage room at the MUFSRF.

8. Results of Human Decedent Trials

Blood impressions for the duration of the trials were deposited onto the decedent donors' skin with each impression assessed to ensure they were of optimal quality, meaning overall patterns and impression details were visible at the time of deposition. The assessment of impression details helped ensure the impressions were of comparable quality upon deposition thus allowing for an assessment of the recoverability of the impressions using three enhancement methods through the early stages of decomposition. A predetermined set of impressions were recovered from each decedent donor at the one-hour (BC1) control interval in the FARL and then daily after the decedents were placed in the field at FROST from the one-day (B01) through ten-day (B10) collection intervals. At each collection interval the blood impressions were photographed insitu on the decent skin prior to enhancement and insitu on the decedent skin after being dye-stained with Amido Black and Hungarian Red. Within an hour after recovery, the lifted Amido Black stained impressions affixed to white BVDA Gellifters® were photographed under normal lighting (NL), the lifted Hungarian Red stained impressions affixed to the black BVDA Gellifters® were photographed under normal (NL) and alternate lighting (AL), and the Zar-Pro™ lifted impressions were photographed under normal (NL) and alternate lighting (AL). The recovered impressions, meaning the impressions lifted with the white and black Gellifters and Zar-Pro™ were photographed again at the MUFSRF one month after recovery. Ratings of impression details (ID) and Fluorescent Intensity (FI) were conducted by two student examiners and two practicing latent print analysts from digital images compiled into data sets based on collection interval.

Recovery of Blood Impressions from Decedent Donors 1 and 2

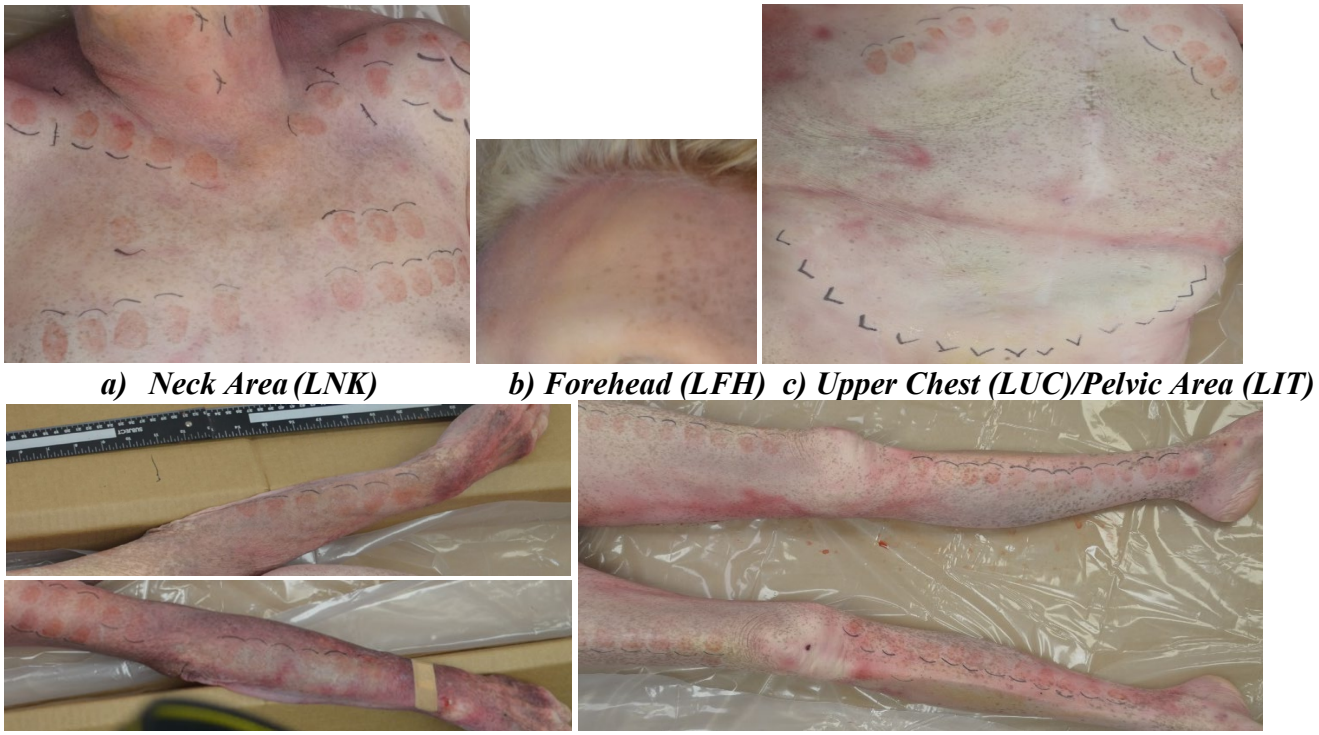
Blood impressions were deposited onto the skin of decedent donors in predesignated locations: the neck area (LNK) which ended up being the neck, shoulders, and upper chest (Figures 33a and 34a), the upper chest area (LUC) which can be more clearly identified as the rib margin (Figures 33c and 34c), the right and left arms (LRA/LLA) (Figures 33d and 34d), and the left and right legs (LRL/LLL) (Figures 33e and 34e). The forehead (LFH) (33b and 34b) was free of any deposited impressions and served as the area of non-impression controls. The skin was already starting to decompose on both decedents at the time of impression deposition. Decedent donor 1 had sores on the skin, with many sores on the legs, and some open wounds and molding areas visible on the neck, abdomen, and both arms. There were areas of skin peeling, as pieces of the epidermal skin was starting to slough off the body and green coloration due to decomposition was visible in the pelvic area and upper legs. Decedent donor 2 had open sores on both arms primarily toward the wrist areas with additional scabbed over sore throughout the body. The skin peeling was more evident on decedent 2 with areas of peeling on the shoulders and lower arms, so much that during the deposition of the blood impressions skin was lifted onto the depositors' thumbs. Green discoloration due to decomposition was visible in the pelvic area, lower legs, and feet. Neither decedent donor was overly hairy with the most hair found on the arms and legs, they also both had fairly light-colored skin which helped in the visualization of the deposited impressions. In total, 260 blood impressions were deposited onto the skin of the decedent donors with 24 impressions deposited in each of the pre-designated areas with 10 additional blood impressions deposited on the upper chest area.



a) Neck Area (LNK) *b) Forehead (LFH)* *c) Upper Chest (LUC)/Pelvic Area (LIT)*

d) Right Arm (LRA) / Left Arm (LLA *e) Right Leg (LRL) / Left Leg (LLL)*

Figure 33: Blood Impressions Deposited on Decedent 1



a) Neck Area (LNK) *b) Forehead (LFH)* *c) Upper Chest (LUC)/Pelvic Area (LIT)*

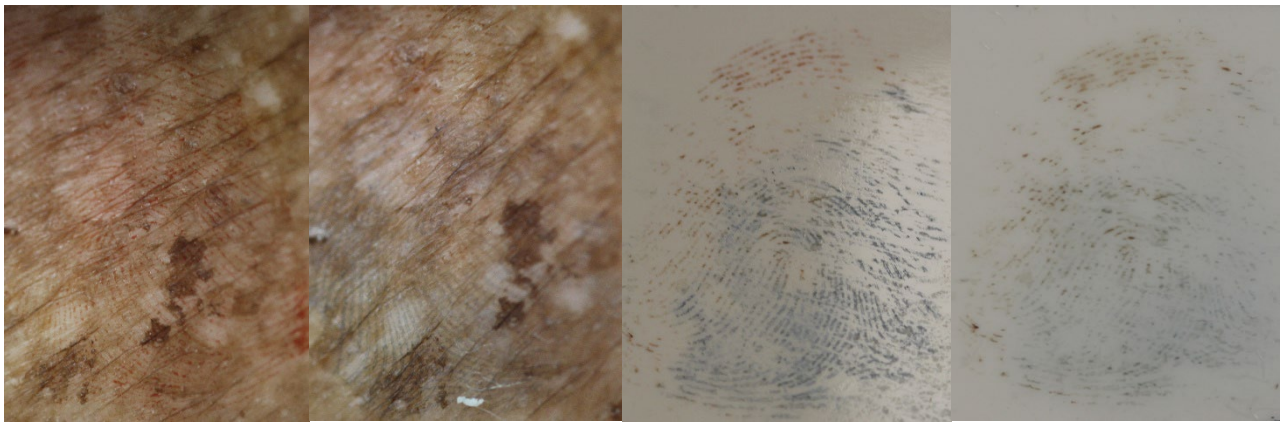
d) Right Arm (LRA) / Left Arm (LLA *e) Right Leg (LRL) / Left Leg (LLL)*

Figure 34: Blood Impressions Deposited on Decedent 2

Blood Control Collection Interval (BC1)

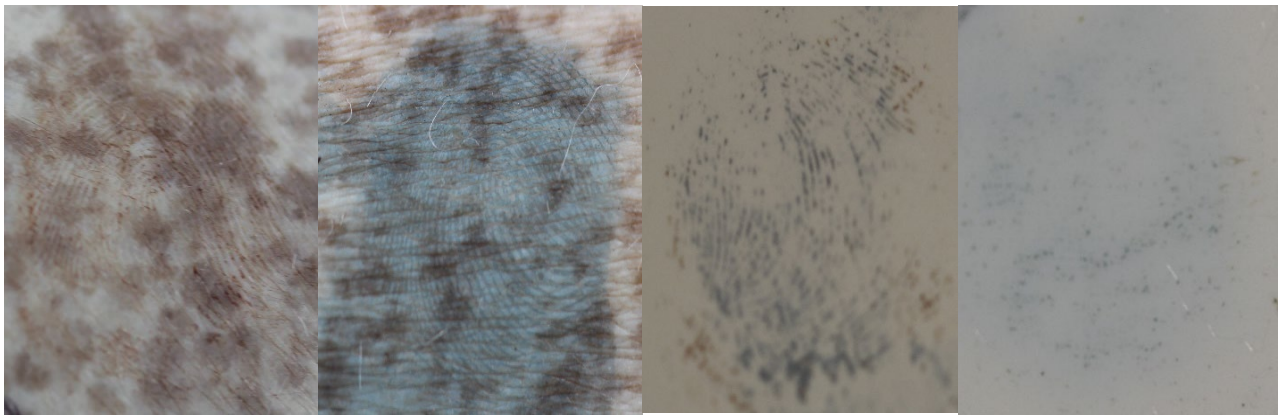
The control impressions for the trials were recovered one-hour after deposition, with all three enhancement methods effective for the enhancement of blood impressions from the decedent skin

(Figures 35-40). At this interval blood impressions were readily visible with impression details on the decedent skin prior to enhancement (Figures 35a, 36a). Amido Black dye-stained impressions were darkened by the stain and visible insitu on the decedent skin (Figures 35b and 36b). The dye-stained impressions were able to be lifted onto the white Gellifters which allowed for the visualization of impression details without the substrate variables of the skin itself impeding with the visualization of impression details (Figure 35c and 36c). However, the dye-stained impressions were faint in some impression areas (Figure 35b) and some of the impression details were lost in the lifting process (Figure 36c). Visualization of the impression details were best when captured one hour after recovery (Figures 35c and 36c) as the details of the dye-stained impressions were lost when left on the Gellifters and photographed one month later (Figures 35d and 36d). Impression details were diffused and lightened when affixed to and aged on the Gellifters, thus photography should be conducted shortly after recovery.



a) Insitu ***b) Insitu Stained*** ***c) Gellifter 1 Hour*** ***d) Gellifter 1 Month***

Figure 35: 1 Hour Decedent 1- Right Arm Amido Black Enhanced Impressions

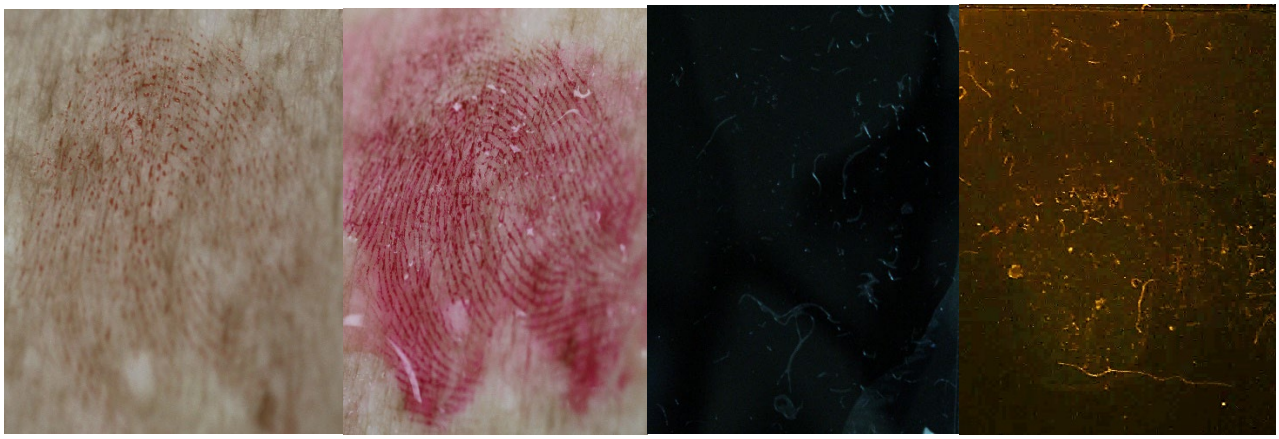


a) Insitu ***b) Insitu Stained*** ***c) Gellifter 1 Hour*** ***d) Gellifter 1 Month***

Figure 36: 1 Hour Decedent 2- Right Leg Amido Black Enhanced Impressions

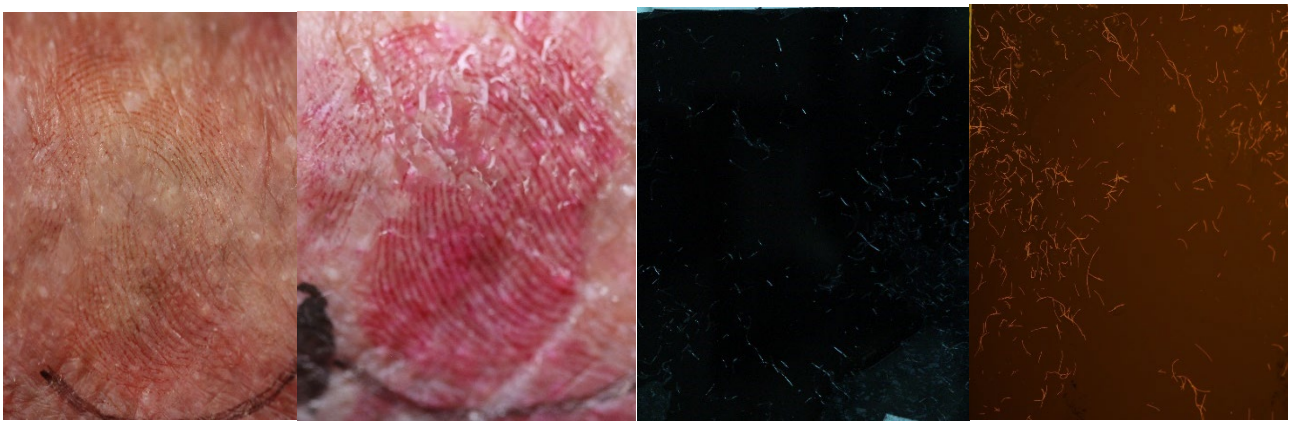
Blood impressions were readily visible with impression details on the decedent skin prior to enhancement (Figures 37a and 38a). Hungarian Red dye-stained impressions were darkened by the stain and visible insitu on the decedent skin (Figures 37b and 38b). The dye-stained impressions were not recovered effectively using the black Gellifters, as only proteinaceous materials were visible on the Gellifters under both normal (Figures 37c and 38c) and alternate lighting (Figures 37d and 38d). The dye-stained proteinaceous materials were fluorescent under alternate lighting, but

impression details were still not visible (Figure 38d). There were also cotton fibers from the enhancement trays that were collected with the Gellifters which can be seen under normal (Figures 37c and 38c) and alternate lighting (Figures 37d and 38d). The pictured Gellifters were taken within an hour after recovery, as with the white Gellifters the proteinaceous materials were less visible a month after recovery.



a) Insitu ***b) Insitu Stained*** ***c) Gellifter N/L*** ***d) Gellifter A/L***

Figure 37: 1 Hour Decedent 1- Left Leg Hungarian Red Enhanced Impressions



a) Insitu ***b) Insitu Stained*** ***c) Gellifter N/L*** ***d) Gellifter A/L***

Figure 38: 1 Hour Decedent 2- Right Arm Hungarian Red Enhanced Impressions

Blood impressions were again readily visible with impression details on the decedent skin prior to enhancement (Figures 39a and 40a). Zar-Pro™ lifted impressions were readily visible under normal (Figure 39b and 40b) and alternate lighting (Figures 39c, 39d, 40c, and 40d). Lifting the impression from the skin substrate onto the white background of the Zar-Pro™ Lifters removes the substrate variables that can affect the visualization of impression details. The lifted impressions were photographed within one hour of impression recovery under both normal (Figure 39b and 40b) and alternate lighting (Figures 39c and 40d). The photographs taken at one hour and one month under normal lighting were the same quality, as impression details affixed onto Zar-Pro™ are not lost or destroyed likely due to the photo fixative embedded within the lifters. The alternate light photos at one hour were still not free of moisture from the Zar-Pro™ Activator thus fluorescence was quenched (Figures 39c and 40c), however by the one month photographs the proteinaceous impressions affixed to lifters were brightly fluorescent (Figures 39d and 40d).

a) Insitu *b) Zar-Pro™ Lift* *c) Zar-Pro™ 1 Hour* *d) Zar-Pro™ 1 Month*

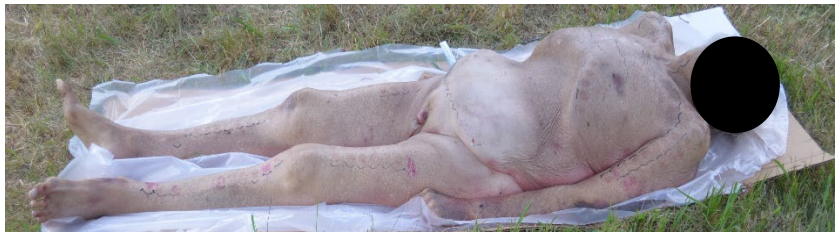
Figure 39: 1 Hour Decedent 1- Neck Area- Zar-Pro™ Enhanced Impressions

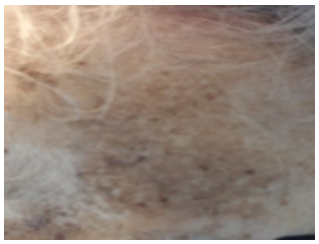
a) Insitu *b) Zar-Pro™ Lift* *c) Zar-Pro™ 1 Hour* *d) Zar-Pro™ 1 Month*

Figure 40: 1 Hour Decedent 2- Left Leg Zar-Pro™ Enhanced Impressions

One-day Collection Interval (B01)

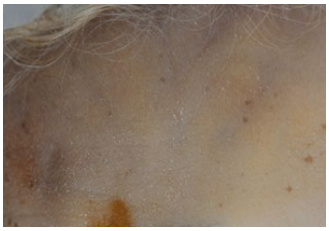
At the one-day collection interval, the decedent skin was still in good condition (Figures 41a and 42b) and the one-day non-impression controls from the decedent foreheads (Figures 41b and 42a) were collected at this interval. There was nothing visible on the white Gellifters under normal lighting for the non-impression control, so they were also visualized under alternate lighting (Figures 43a and 44a) which allowed for slightly fluorescent proteinaceous materials to be visible. There was also nothing visible on the black Gellifters under normal lighting, but fluorescent proteinaceous materials were visible under alternate lighting (Figure 43b and 44b). The Zar-Pro™ Lifters did have some proteinaceous materials visible on the white background of the Lifter under normal lighting (Figures 43c and 44c) and there was mildly fluorescent contamination from proteinaceous materials under alternate lighting (Figures 43d and 44c).





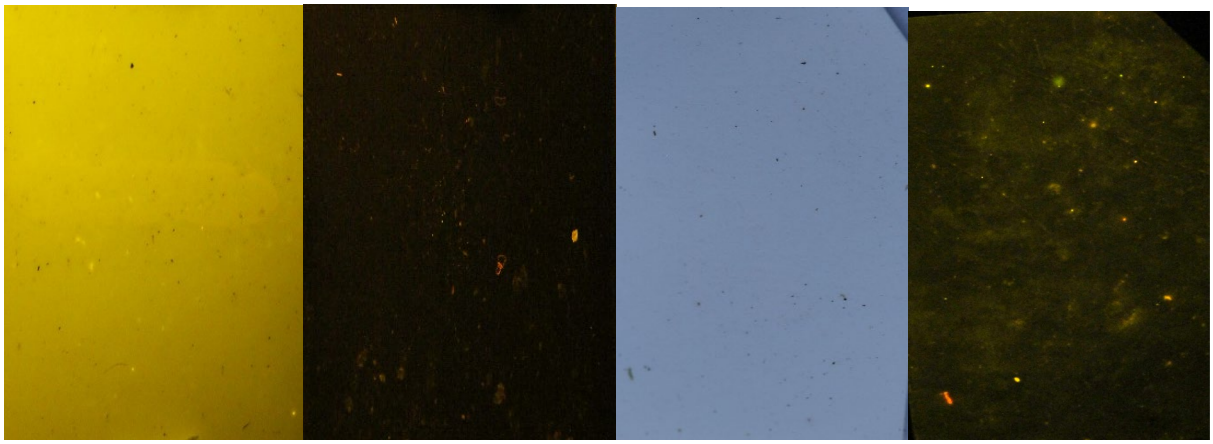
a) Body *b) Forehead*

Figure 41: Decedent Donor 1 – 1 Day Collection Interval



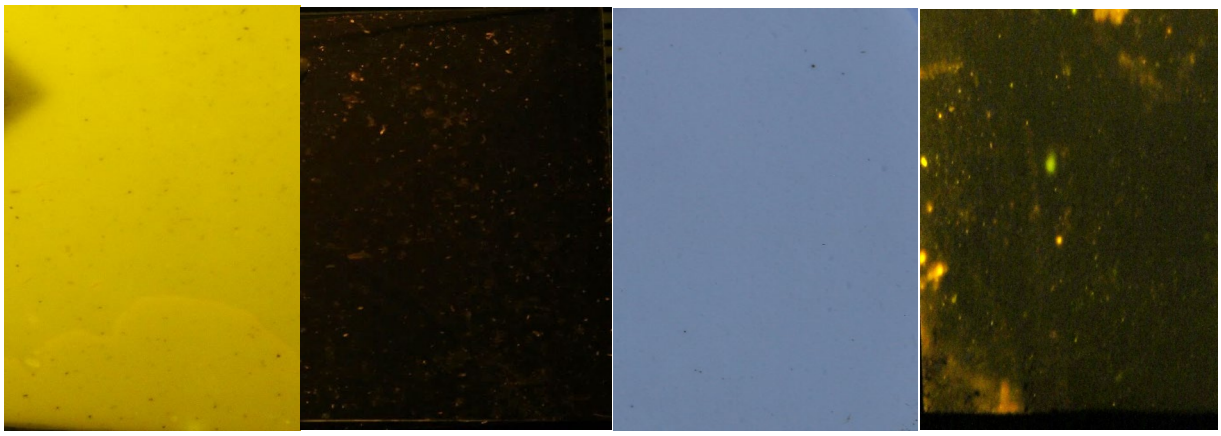
a) Forehead *b) Body*

Figure 42: Decedent Donor 2 – 1 Day Collection Interval



a) White Gellifter *b) Black Gellifter* *c) Zar-Pro™ 1 Hour* *d) Zar-Pro™ 1 Month*

Figure 43: Day1 Decedent 1- Forehead Non-Impression Controls



a) White Gellifter *b) Black Gellifter* *c) Zar-Pro™ 1 Hour* *d) Zar-Pro™ 1 Month*

Figure 44: Day 1 Decedent 2- Forehead Non-Impression Controls

At the one-day collection interval the blood impressions were still readily visible on the skin of the decedents prior to enhancement (Figures 45a, 46a, and 47a). Flies surrounded both decedents, with adult flies frequenting the body orifices, such as the eyes, mouth, and genital areas. There were also some spiders seen crawling on and around the bodies. The areas of green discoloration and mold spots were growing on both decedents, donor 1 had more pronounced skin peeling and areas of slippage on the right arm. Donor 2 started to bloat in the neck with skin peeling now noted on the forehead and the previously scabbed sores were now uncovered. The Amido black dye-stained impressions were visible insitu on the decedents skin (Figure 45b) and the white Gellifters were able to recover some visible ridge details from the stained impressions (Figure 45c). As with the one-hour control impressions the white Gellifters were not overly successful in the recovery of the insitu stained impression as ridge details were lost in transfer to the Gellifter. The Hungarian Red dye-stained impressions were also readily visible insitu on the decedent skin (Figure 46b) but only some proteinaceous materials were visible on the black background of the Gellifters under both normal and alternate lighting (Figure 46c). The application of the liquid dye-stains and de-staining rinse onto the skin substrate also resulted in inconsistent staining amongst the blood impressions, so some areas were stained adequately whereas other areas were only lightly stained. The Zar-Pro™ lifted impressions were readily visible under normal lighting (Figure 47b) on the white background of the lifter. The Zar-Pro™ lifted impressions were further enhanced by visualization under alternate lighting (Figure 47c) one month after recovery showing a brightly fluorescent impression on the darkened background of the lifter.

a) Insitu ***b) Insitu Stained*** ***c) Gellifter***

Figure 45: Day 1 Decedent 1- Right Arm Amido Black Enhanced Impressions

a) Insitu *b) Insitu Stained* *c) Gellifter*

Figure 46: Day 1 Decedent 2- Right Arm Hungarian Red Enhanced Impressions

a) Insitu *b) Normal Lighting (NL)* *c) Alternate Lighting (AL)*

Figure 47: Day 1 Decedent 2 – Left Leg Zar-Pro™ Enhanced Impressions

Two-day Collection Interval (B02)

At the two-day collection interval, there were more insects on and around the bodies, specifically flies, ants, bees, and spiders. The decedents eyes were beginning to dry out with the areas of decomposition on the skin growing and darkening in color. Lacerated skin areas were opening and becoming more abundant on the bodies. Donor 1 (Figure 48) had signs of skin beginning to peel as the epidermal layer was starting to separate from the dermal skin, this was most notable on the right shoulder area. Discoloration of the body was most pronounced on the left arm and hand, along with the right hand and hip area. Donor 2 (Figure 49) had early stages of skin peeling with discoloration most pronounced on the arms, shoulders, neck and head and visible bloating in the face and neck. Although the skin was darker and discolored due to the early signs of decomposition, impression details were still visible insitu on the decedent skin prior to enhancement (Figures 50a, 51a. and 52a). The Amido black dye-stained impressions were visible insitu on the decedents skin (Figure 50b) and the white Gellifters were able to recover some of the impression details of the stained impressions (Figure 50c), but the overall quality of recovered impressions was decreasing. The Hungarian Red dye-stained impressions were also readily visible insitu on the

decedent skin (Figure 51b) but there were no visible proteinaceous materials visible on the black background of the Gellifters under normal or alternate lighting (Figure 51c). Zar-Pro™ lifted impressions were readily visible under normal lighting (Figure 52b) on the white background of the lifter, improving visualization of impression details as the lifting the impression from the decedent skin removes the substrate variables associated with the skin which were more pronounced due to artifacts of decomposition. The Zar-Pro™ lifted impressions were further enhanced by visualization under alternate lighting (Figure 52c) which allows for the visualization of a brightly fluorescent impression on the darkened background of the lifter.

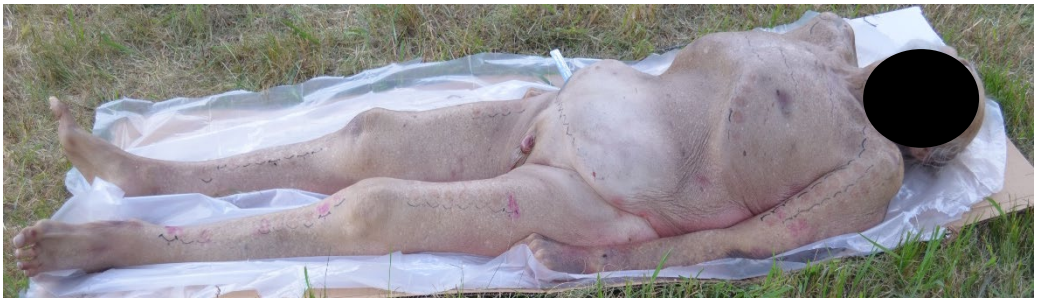


Figure 48: Decedent Donor 1 – Day 2 Collection Interval

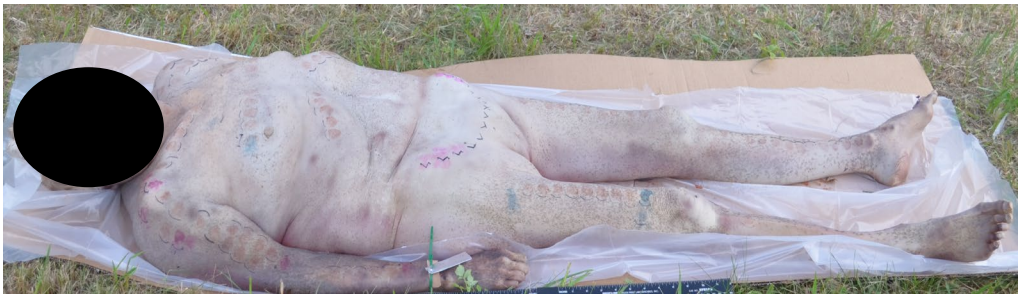
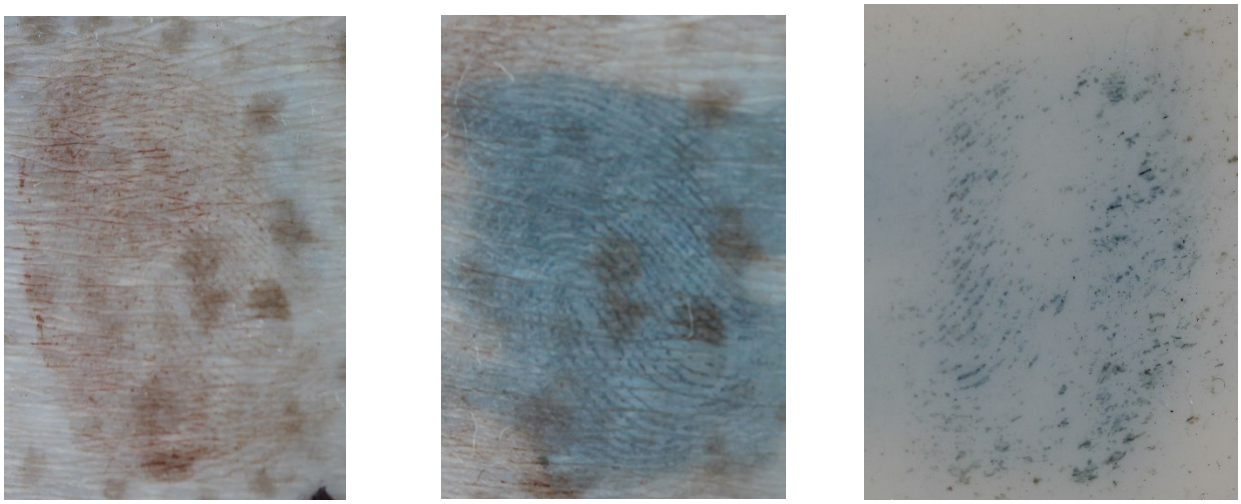
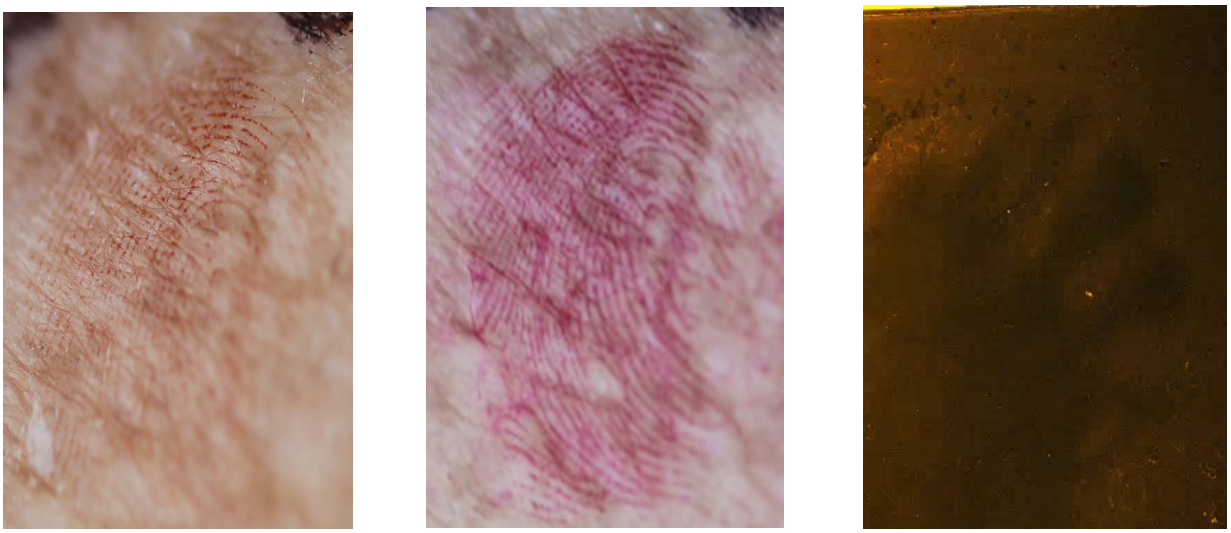


Figure 49: Decedent Donor 2 – Day 2 Collection Interval



a) Insitu ***b) Insitu Stained*** ***c) Gellifter***

Figure 50: Day 2 Decedent 2- Neck Area Amido Black Enhanced Impressions



a) Insitu *b) Insitu Stained* *c) Gellifter*

Figure 51: Day 2 Decedent 1- Left Arm Hungarian Red Enhanced Impressions

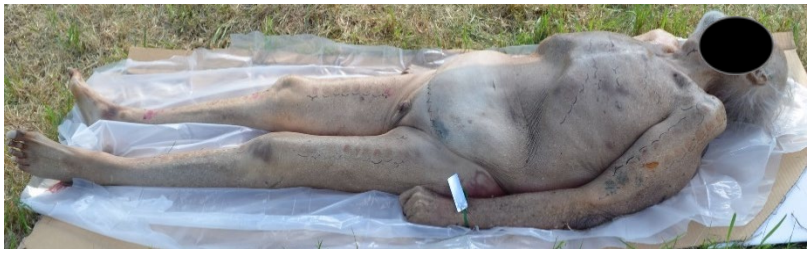
a) Insitu *b) Normal Lighting (NL)* *c) Alternate Lighting (AL)*

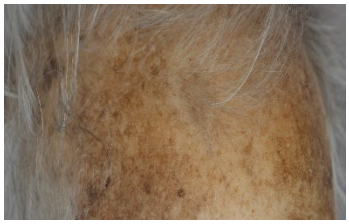
Figure 52: Day 2 Decedent 1- Right Leg Zar-Pro™ Enhanced Impressions

Three-day Collection Interval (B03)

At the three-day collection interval, there were substantially more insects on and around the bodies from the previous collection interval. There were numerous adult flies mostly around the body orifices with egg masses in the eyes, nose, and mouth. There were also bees, ants, and several spiders on and around the bodies. The decedents were leaking body fluids around the arms and hands, legs and feet, and pelvic areas with skin lacerations throughout the body. Donor 1 (Figure 53a) had increasing areas of skin slippage and darkening areas of discoloration from decomposition in the arms, neck, and chest areas. Donor 2 (Figure 54b) had darkening areas of decomposition in the arms, inner thighs, and neck area. The three-day non-impression controls were collected from the decedent foreheads (Figures 53b and 54a). There was nothing visible on the white Gellifters under normal lighting for the non-impression controls, so they were visualized under alternate lighting (Figures 55a and 56a) which allowed for slightly fluorescent proteinaceous materials to be visible. There was also nothing visible on the black Gellifters under normal lighting, but fluorescent proteinaceous materials were scattered throughout the Gellifter and visible under alternate lighting

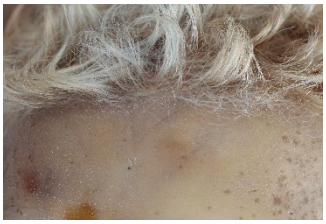
(Figures 55b and 56b). The Zar-Pro™ Lifters did have some proteinaceous materials scattered throughout the Lifters which were visible under normal lighting (Figures 55c and 56c) and proteinaceous contamination was visible under alternate lighting (Figures 55d and 56d).

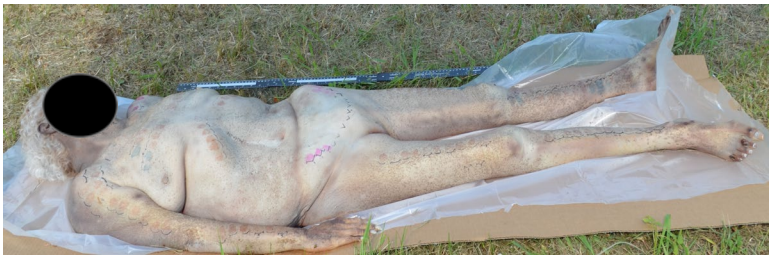




a) Body ***b) Forehead***

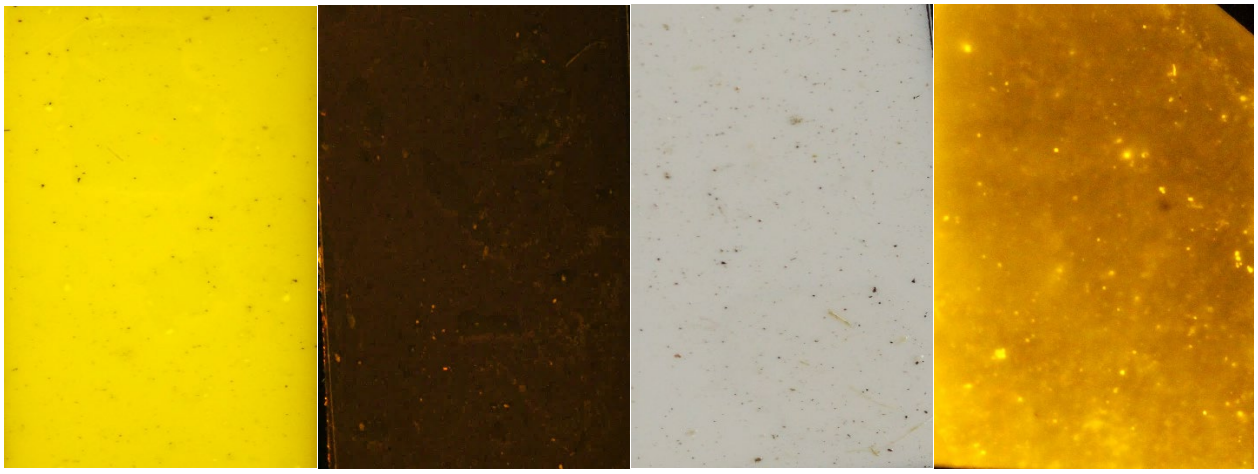
Figure 53: Decedent Donor 1 – Day 3 Collection Interval





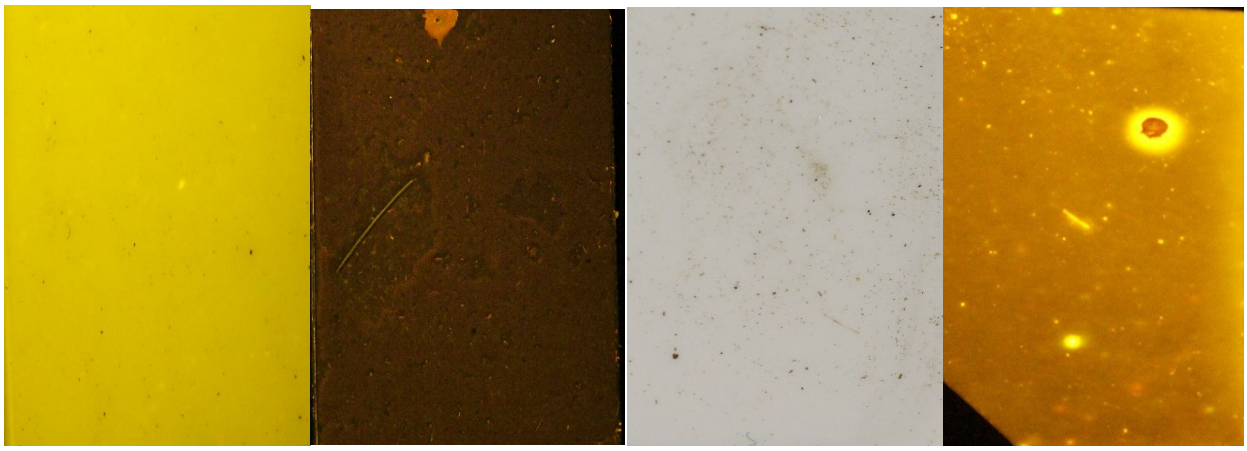
a) Forehead ***b) Body***

Figure 54: Decedent Donor 2 – Day 3 Collection Interval



a) White Gellifter ***b) Black Gellifter*** ***c) Zar-Pro™ NL*** ***d) Zar-Pro™ AL***

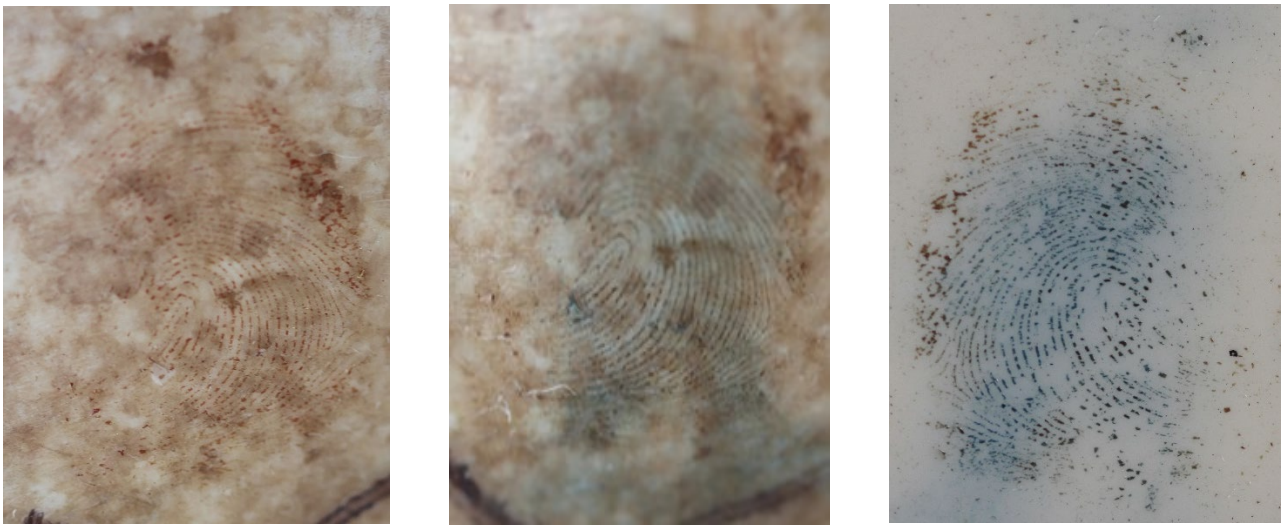
Figure 55: Day 3 Decedent 1- Forehead Non-Impression Controls



a) White Gellifter *b) Black Gellifter* *c) Zar-Pro™ NL* *d) Zar-Pro™ AL*

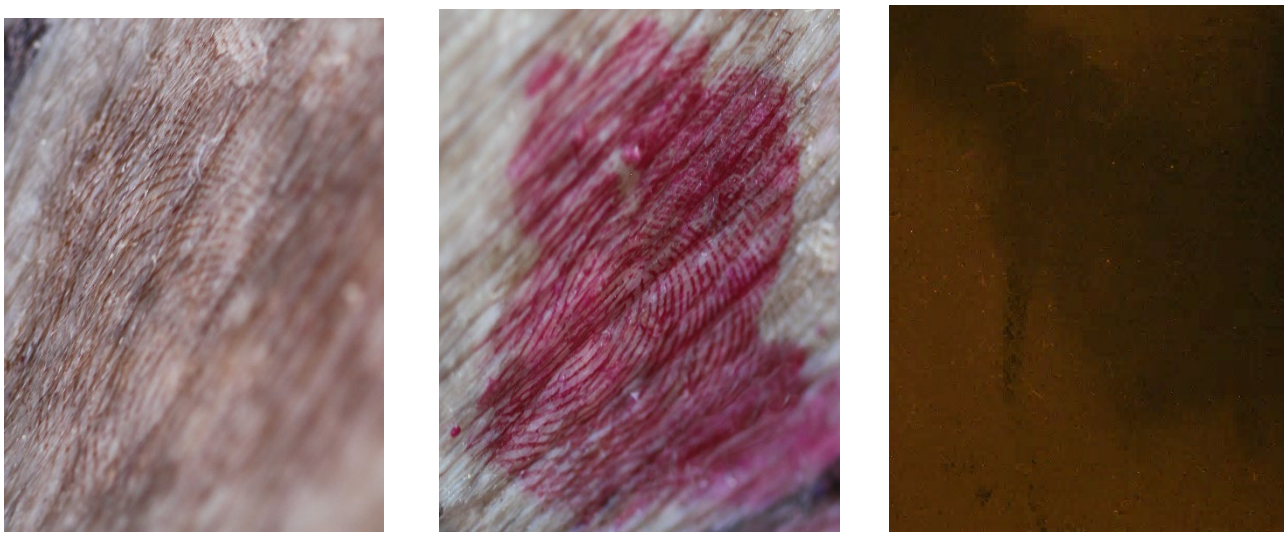
Figure 56: Day 3 Decedent 2- Forehead Non-Impression Controls

Blood impressions were still visible insitu on decedent skin prior to enhancement but were becoming fainter with impression details not as readily visible (Figures 57a, 58a, and 59a). Amido black dye-stained impressions were visible insitu on the decedents skin (Figure 57b) and the white Gellifters were still able to recover some of the impression details of the stained impressions (Figure 57c). Hungarian Red dye-stained impressions were also readily visible insitu on the decedent skin (Figure 58b) but there were still no proteinaceous materials visible on the black background of the Gellifters under normal or alternate lighting (Figure 58c). Zar-Pro™ lifted impressions were still readily visible under normal lighting (Figure 59b) on the white background of the lifter, and further enhanced by visualization under alternate lighting (Figure 59c) which allows for the visualization of a brightly fluorescent impressions on the darkened background of the Lifter.



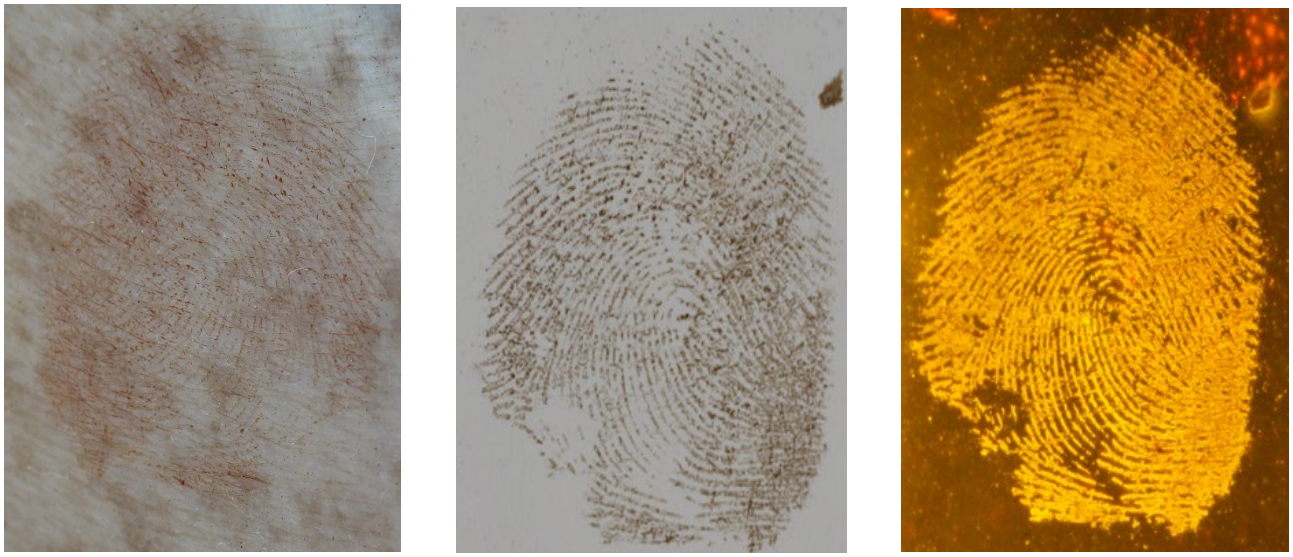
a) Insitu *b) Insitu Stained* *c) Gellifter*

Figure 57: Day 3 Decedent 1- Right Arm- Amido Black Enhanced Impressions



a) Insitu *b) Insitu Stained* *c) Gellifter*

Figure 58: Day 3 Decedent 2- Right Arm Hungarian Red Enhanced Impressions



a) Insitu *b) Normal Lighting (NL)* *c) Alternate Lighting (AL)*

Figure 59: Day 3 Decedent 2- Left Arm Zar-Pro™ Enhanced Impressions

Four-day Collection Interval (B04)

At the four-day collection interval, insect activity was increasing with adult flies swarming around the orifices, many of which are dropping eggs in the eyes, nose, mouth, and pelvic areas. Maggots were now emerging from the orifices, and from under the epidermal skin layer via the skin lacerations primarily seen under the arms and on the body torsos. Skin discoloration is progressing on both decedents (Figures 60 and 61), they are also leaking biofluids and the skin lacerations are increasing in size and abundance. Blood impressions were still readily visible on the skin of the decedents prior to enhancement but they were increasingly becoming fainter (Figures 62a, 63a, and 64a). There were precipitation droplets on the donors, notably on the leg of Donor 2 due to rainfall from the previous evening. There was noticeable contamination on some of the blood impressions from insect activity, particularly small pools of fly vomit (Figures 63a and 64a). Amido Black dye-

stained impressions were visible insitu on the decedents skin (Figure 62b) and the white Gellifters were again able to recover some of the impression details of the stained impressions (Figure 62c). The Hungarian Red dye-stained impressions were also readily visible insitu on the decedent skin (Figure 63b) but only some proteinaceous materials were visible on the black background of the Gellifters under alternate lighting (Figure 63c) with fiber contamination visible on the Gellifter. Zar-Pro™ lifted impressions were readily visible under normal lighting (Figure 64b) but some of the Lifters had fainter impressions details than were previously noted. However, due to the fluorescent properties of the Zar-Pro™ Lifters even the faint impression details were brightly fluoresced when visualization under alternate lighting (Figure 64c).

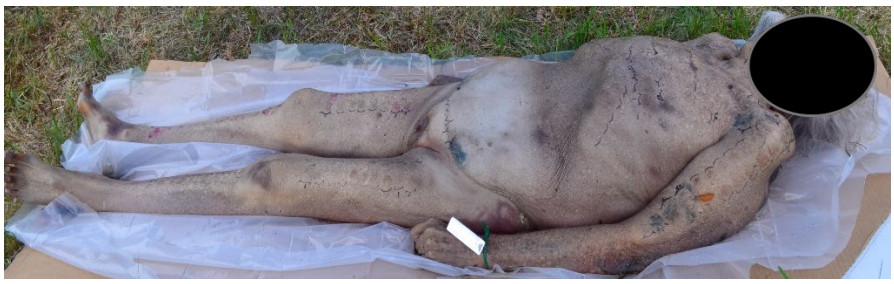
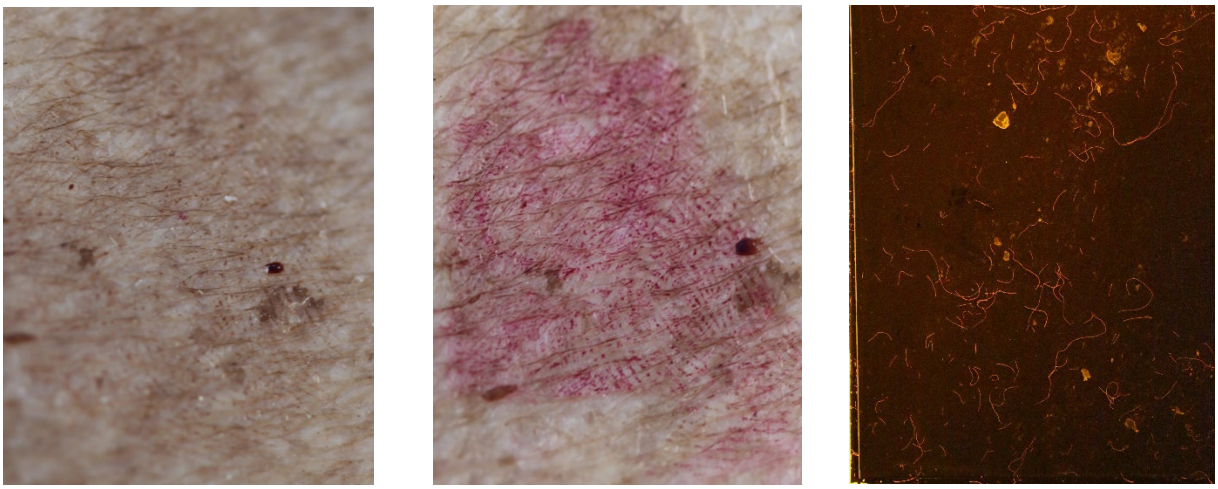


Figure 60: Decedent Donor 1 – Day 4 Collection Interval

Figure 61: Decedent Donor 2 – Day 4 Collection Interval

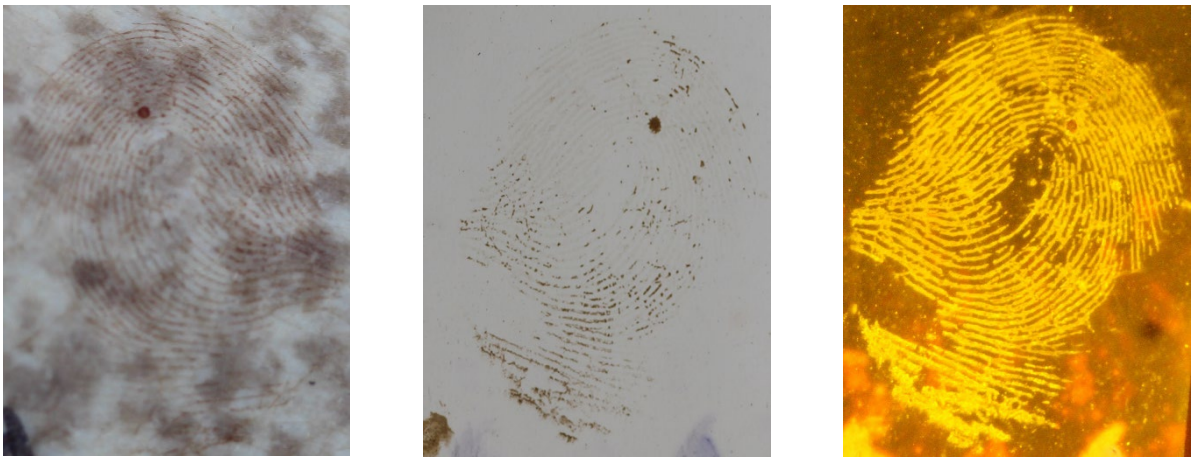
a) Insitu ***b) Insitu Stained*** ***c) Gellifter***

Figure 62: Day 4 Decedent 2- Right Leg Amido Black Enhanced Impressions



a) Insitu *b) Insitu Stained* *c) Gellifter*

Figure 63: Day 4 Decedent 1- Left Leg Hungarian Red Enhanced Impressions



a) Insitu *b) Normal Lighting (NL)* *c) Alternate Lighting (AL)*

Figure 64: Day 4 Decedent 2- Left Leg Zar-Pro™ Enhanced Impressions

Five-day Collection Interval (B05)

At the five-day collection interval, the decedent donors were advancing in decomposition, the epidermal skin was drying out and separating from the dermal skin, maggots were abundant beneath the epidermal skin layer and in any open wounds. The adult flies were still swarming the body and other insects were present on and around the body such as ants, bees, beetles, and spiders. Evidence of the insect movements, primarily from the flies which were seen in abundance on the decedents skin, in some cases this contamination was on top of the impression area (Figures 69-71). The decedents were still leaking biofluids, but the skin now appeared lighter than in the previous collection intervals, this was due to the drying of the epidermis which makes the discoloration of the decedents less pronounced. Donor 1 (Figures 65) had an abundance of maggots under the epidermal layer some of which were lifting the impression area from the dermal skin (Figure 69a). This was more pronounced on the arms, neck, chest, pelvic areas, and on the forehead, whereas the leg skin was not yet separated. Donor 2 (Figure 66) also had maggots under the epidermal layer with some eating through the skin or crawling out of wounds to emerge from between the epidermal and dermal skin layers. As seen with donor 1, the maggot activity was less pronounced on the decedent

legs. The five-day non-impression controls were collected from the decedent foreheads (Figures 65b and 66a). There was nothing visible on the white Gellifters non-impression controls under normal lighting, but there were some proteinaceous materials visualized under alternate lighting (Figures 67a and 68a). There was also nothing visible on the black Gellifters under normal lighting, but fluorescent proteinaceous materials were visible on the Gellifter under alternate lighting (Figures 67b and 68b). It was noted that the epidermal skin from the decedents was lifting onto the Gellifters, thus the use of Gellifters was no longer a viable option as the epidermal skin layer containing the insitu stained impression could be damaged in the lifting process. The Zar-Pro™ Lifters had visible proteinaceous materials and decomposition residue on the Lifter which was visible under normal (Figure 67c and 68c) and alternate lighting (Figure 67d and 68d). The background contamination due to insect movement and activity could impede visualization of impression details on the Zar-Pro™ Lifts when covering the impression area.

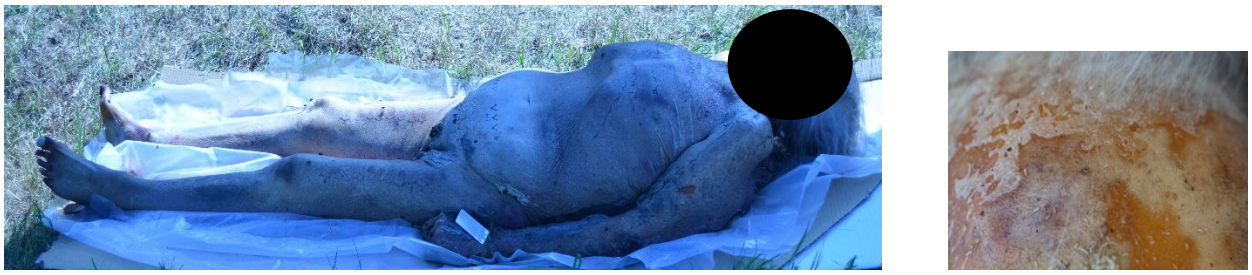
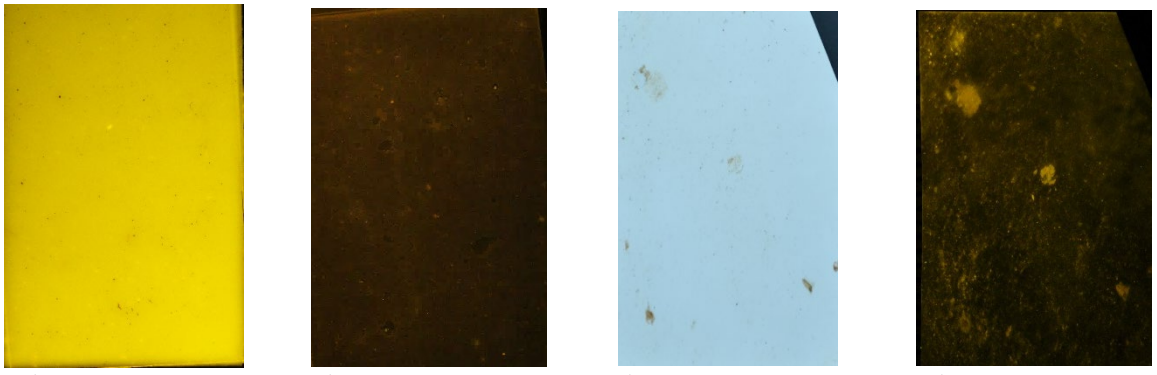


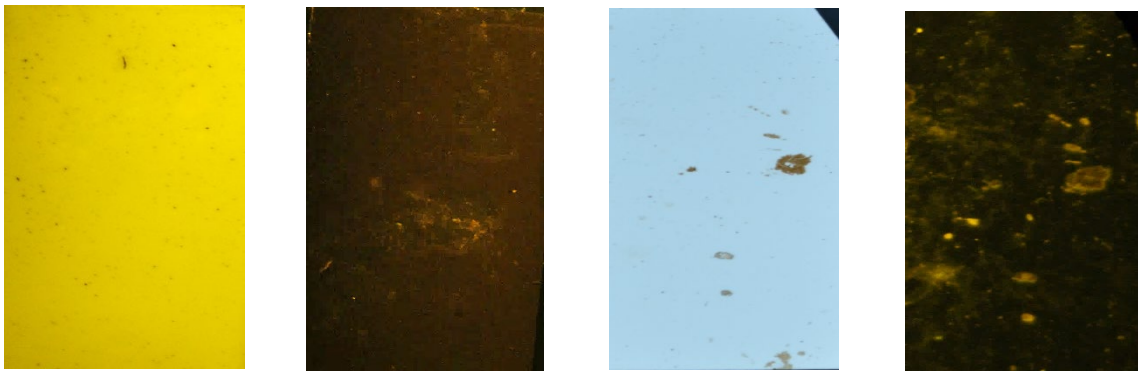
Figure 65: Decedent Donor 1 – Day 5 Collection Interval

Figure 66: Decedent Donor 2 – Day 5 Collection



a) White Gellifter ***b) Black Gellifter*** ***c) Zar-Pro™ NL*** ***d) Zar-Pro™ AL***

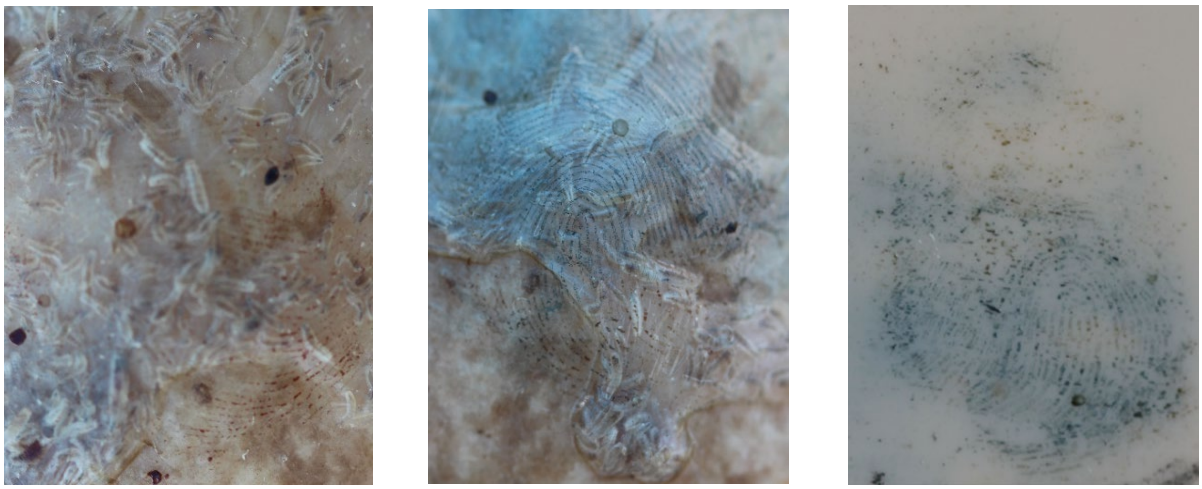
Figure 67: Day 5 Decedent 1- Forehead Non-Impression Controls



a) White Gellifter *b) Black Gellifter* *c) Zar-Pro™ NL* *d) Zar-Pro™ AL*

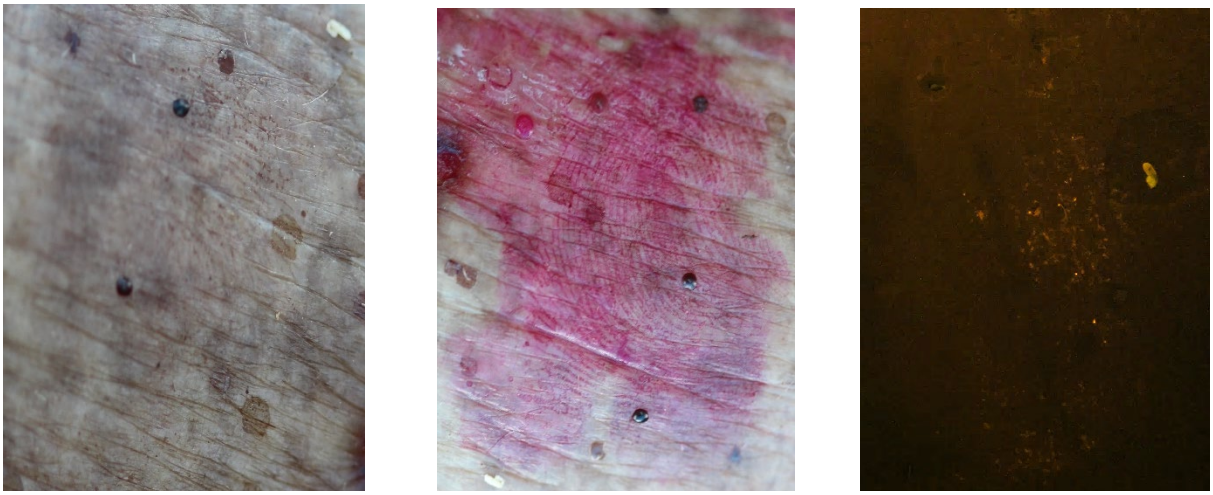
Figure 68: Day 5 Decedent 2- Forehead Non-Impression Controls

Blood impressions were still visible insitu on decedent skin prior to enhancement but were becoming fainter with impression details not as readily visible as in the prior collection intervals (Figures 69a, 70a, and 71a). Furthermore, the maggots between the epidermal and dermal skin layers were very active and made impression details difficult to visualize insitu. Even with the maggots moving between the skin layers, Amido Black dye-stained impressions were visible insitu on the decedent skin (Figure 69b). The white Gellifters were able to lift some of the dye-stained impression details but again impression details were lost in the transfer (Figure 69c). Hungarian Red dye-stained impressions were also visible insitu on the decedent skin (Figure 70b) but only some proteinaceous materials were visible on the black background of the Gellifters under normal and alternate lighting (Figure 70c). Zar-Pro™ lifted impressions were readily visible under normal lighting (Figure 71b) on the white background of the lifter, and further enhanced by visualization under alternate lighting (Figure 71c) which allows for the visualization of a brightly fluorescent impressions on the darkened background of the lifter. Many of the impressions were contaminated by flies with vomit stains found all over the decedent skin even over the impressions, as well as other markings from the fly movements primarily caused by their legs touching biofluids. This was seen in all the enhancement methods, but the Zar-Pro™ Lifters have a high affinity for proteinaceous materials and can lift the same impression more than once. On one specifically contaminated impression the Zar-Pro™ Lifters removed some of the contamination from the flies on the first lift (Figure 72a) allowing for better visualization of the impression details on the second lift (Figure 72b).



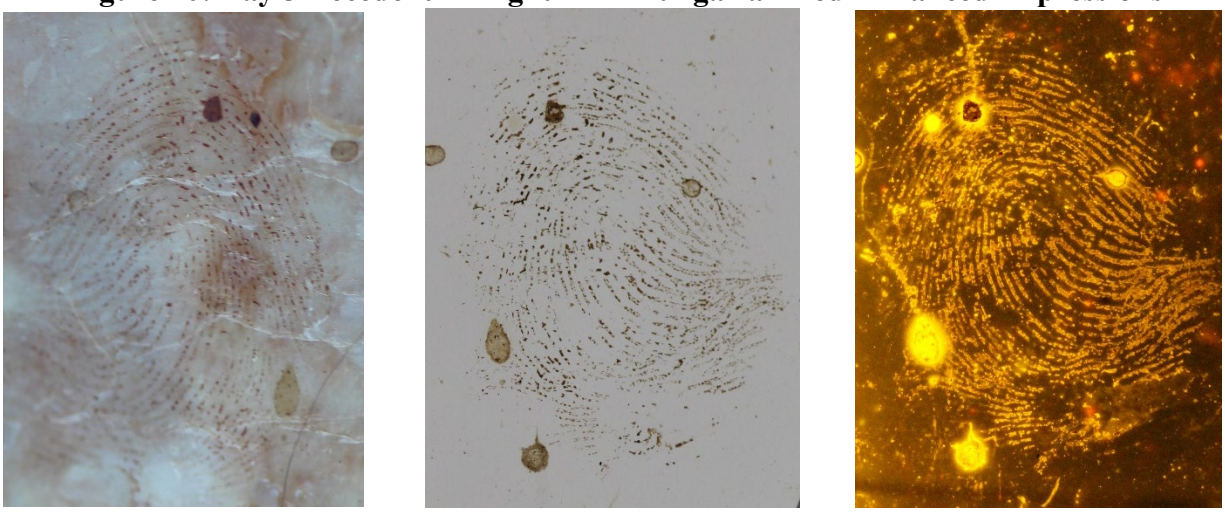
a) Insitu *b) Insitu Stained* *c) Gellifter*

Figure 69: Day 5 Decedent 1- Right Arm Amido Black Enhanced Impressions



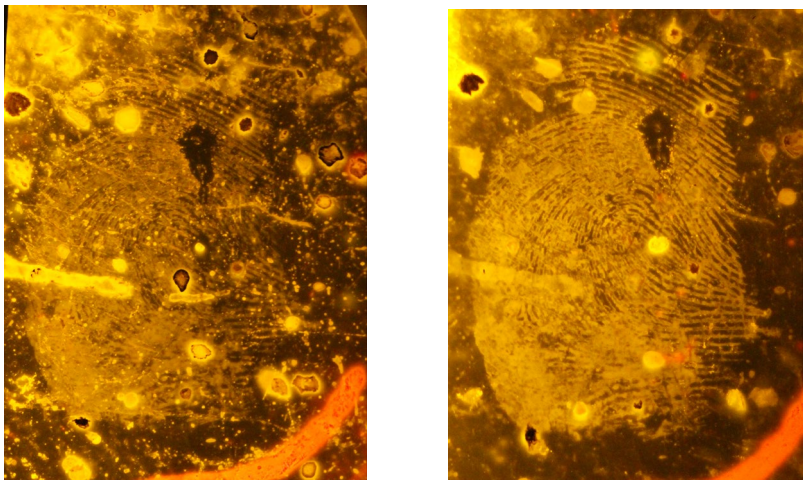
a) Insitu *b) Insitu Stained* *c) Gellifter*

Figure 70: Day 5 Decedent 2- Right Arm Hungarian Red Enhanced Impressions



a) Insitu *b) Normal Lighting (NL)* *c) Alternate Lighting (AL)*

Figure 71: Day 5 Decedent 1- Neck Area Zar-Pro™ Enhanced Impressions



a) First Lift *b) Second Lift*

Figure 72: Day 5 Decedent 2- Left Leg Zar-Pro™ Enhanced Impressions

Six-day Collection Interval (B06)

At the six-day collection interval, maggots were now covering the entire body, both above and below the epidermal layer. There were still adult flies swarming the body, along with the presence of other insects such as bees and beetles. The maggots were abundant between the layers of skin and were also starting to eat through the epidermal layer, leaving small holes in the skin. Both decedents were still leaking biofluids and had complete or near complete separation of the epidermal and dermal layers to include the leg areas. Decedent 2 (Figure 72) was not decomposing as rapidly as Decedent 1 (Figure 73), which had completely lost the epidermal skin layer on the arms, upper body, and face with the exposed dermal layer now a dark brown color and covered in a greasy oil residue. As the epidermal layer continued to separate from the dermal layer, the impression areas were still able to be located due to the detectable presence of a blood, although impression details were increasingly difficult to visualize as the epidermal layer was removed (Figure 75a, 76a, and 77a). There was even more contamination on the blood impressions from insect activity, particularly the small pools of fly vomit and smaller prints made by fly movements. The Amido black dye-stained impressions were not visible insitu on the decedents skin (Figure 75b), but some staining of proteinaceous materials was present and the white Gellifters were able to lift some of the stained materials (Figure 75c). As the epidermal skin was naturally separating from the dermis, the epidermal skin was cut from the decedent to better visualize impression details which were still not readily visible insitu (Figure 75d). The Hungarian Red dye-stained impressions were partially visible insitu on the decedent skin (Figure76b), but proteinaceous materials were not visible on the black background of the Gellifters under normal or alternate lighting. Therefore, the Hungarian Red dye-stained impressions were visualized insitu on the decedent skin using alternate lighting (Figure 76c). The fluorescent capabilities of Hungarian Red did not necessarily improve visualization of impression details as most of the insitu dye-stained impressions were fluorescent but had a lot of background staining which hindered visualization, even when visualized using alternate lighting. Zar-Pro™ lifted impressions were barely visible under normal lighting (Figure 77b) with only the proteinaceous materials from the flies visible on the Lifters. Even though impression details were not visible under normal lighting, brightly fluorescent impression details were lifted and could be visualized under alternate lighting (Figures 77c and d). The first lift of an impression was covered with contamination from flies and other insects (Figure 77c); therefore, a

second lift was taken from the original blood impressions with the second lift showing less contamination over the impression making the impression details more visible (Figure 77d). The ability of the Zar-Pro™ Lifters to be used multiple times on the same impression increases their versatility of use and can help improve the visualization of impression details by removing excessive proteinaceous materials that may hinder visualization.

Figure 73: Decedent Donor 1 – Day 6 Collection

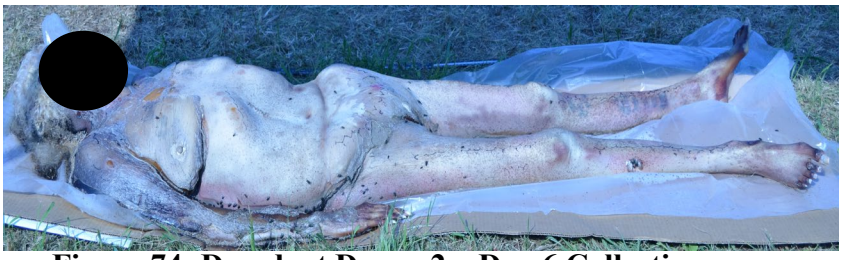
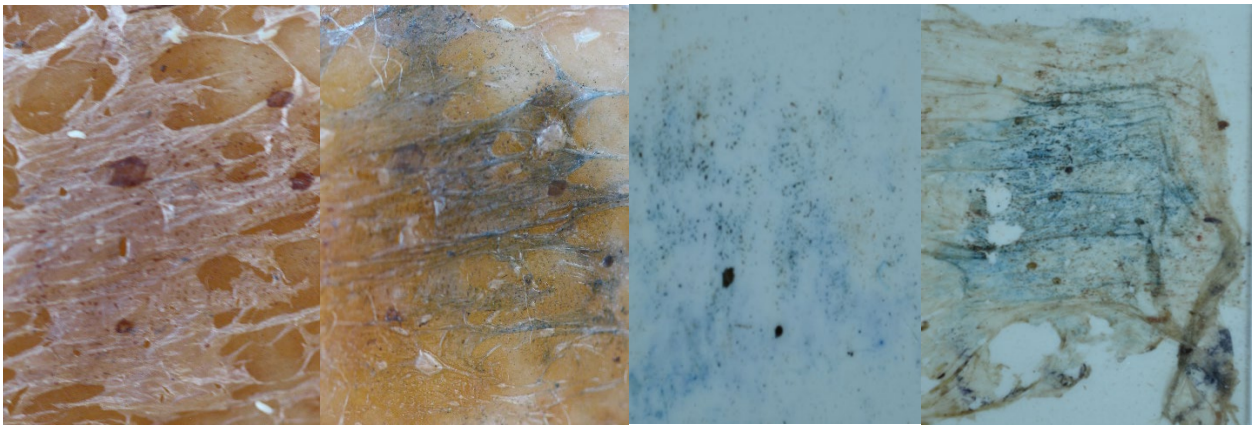
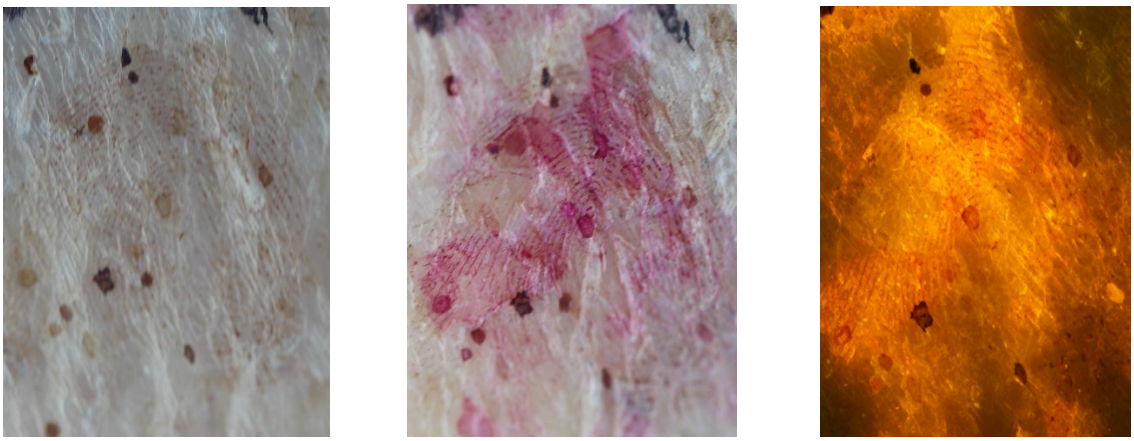


Figure 74: Decedent Donor 2 – Day 6 Collection



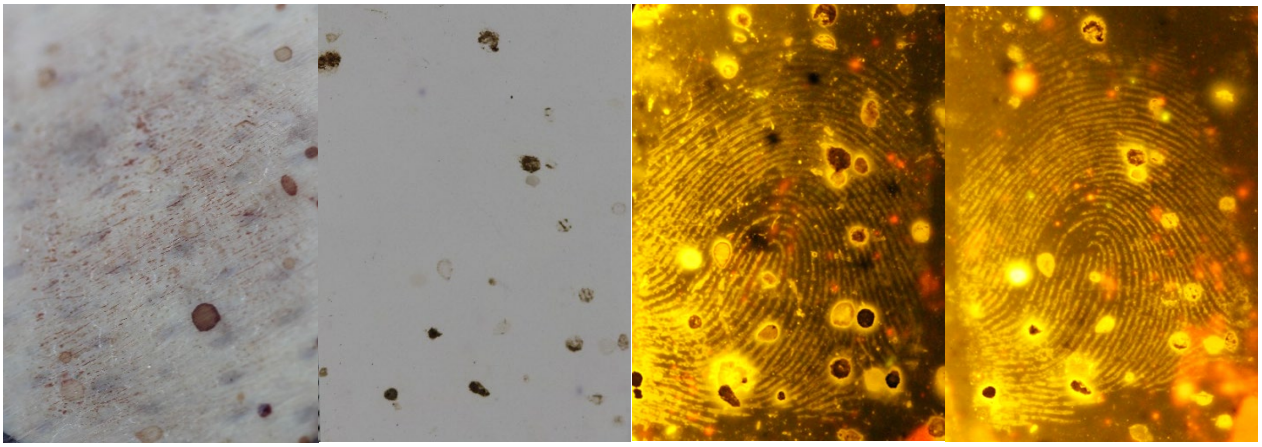
a) Insitu ***b) Insitu Stained*** ***c) Gellifter*** ***d) Stained Epidermis***

Figure 75: Day 6 Decedent 1- Right Arm Amido Black Enhanced Impressions



a) Insitu *b) Insitu Stained* *c) Insitu Stained*

Figure 76: Day 6 Decedent 1- Left Leg Hungarian Red Enhanced Impressions



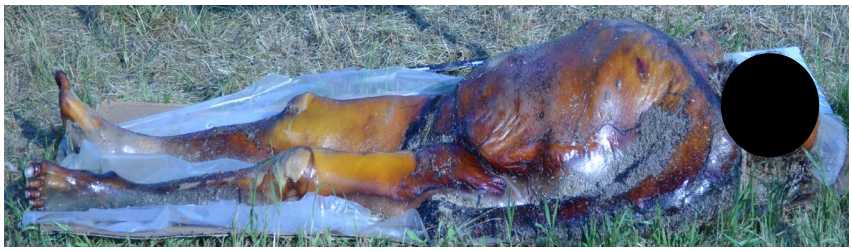
a) Insitu *b) Zar-Pro™* *c) Zar-Pro™ 1st Lift* *d) Zar-Pro™ 2nd Lift*

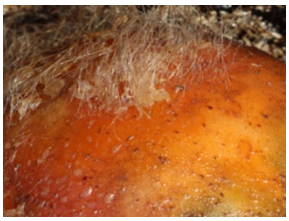
Figure 77: Day 6 Decedent 2- Left Leg- Zar-Pro™ Enhanced Impressions

Seven-day Collection Interval (B07)

At the seven-day collection interval, maggots were still abundant, and the adult flies were still swarming around the bodies. Decedent donor 1 was rapidly advancing in decomposition with noticeable bloat primarily seen on the upper body to include the pelvis, abdomen, chest, and face. The epidermal skin layer was no longer present on most of the body, which exposed the dermal layer which is a dark brown color and covered in a greasy oil residue (Figure 78a). Decedent donor 2 had most of the body still covered in dried epidermal skin with lots of maggot activity between the epidermal and dermal layers. The decedents face, upper body, arms, and genital area were beginning to darken with the skin becoming greasy as the epidermal layer peeled away (Figure 79b). The detection of blood impressions was becoming more difficult on the skin, as the epidermal skin was separating from the dermal layer (Figures 83a, 84a, and 85a). Some of the deposited blood impressions on the epidermal skin penetrated the dermal layer with the dark coloration of blood visible on the dermal skin, although often difficult to visualize (Figure 82a). The seven-day non-impression controls were also collected from the decedent foreheads (Figures 78b and 80a). Only Zar-Pro™ was used on Decedent donor 1 due to the advanced stages of decomposition, thus the Gellifter controls were no longer used on this decedent. The Lifters had visible proteinaceous materials on the white background when visualized under normal lighting (Figure 80a) and

fluoresced when visualized under alternate lighting (Figure 80b). There was a lot of background contamination on the Zar-Pro™ Lifters from Decedent 1 at this stage of decomposition. As for decedent donor 2 non-impression controls, there were visible proteinaceous materials on the white Gellifters under normal and alternate lighting (Figure 81a). There was nothing visible on the black Gellifters under normal lighting, but some fluorescent proteinaceous materials were visible on the Gellifter under alternate lighting (Figure 81b). The Zar-Pro™ Lifters had lots of visible proteinaceous materials as a result of decomposition on the background of the Lifter under normal (Figure 81c) and alternate lighting (Figure 81d). Thus, it is readily apparent now that background contamination because of decomposition and insect activity will impede visualization of impression details on the Zar-Pro™ lifts.



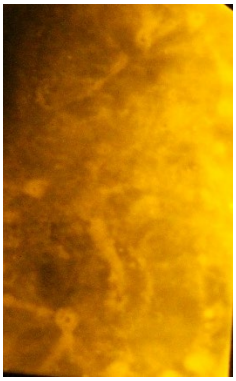


a) Body ***b) Forehead***

Figure 78: Decedent Donor 1 – Day 7 Collection

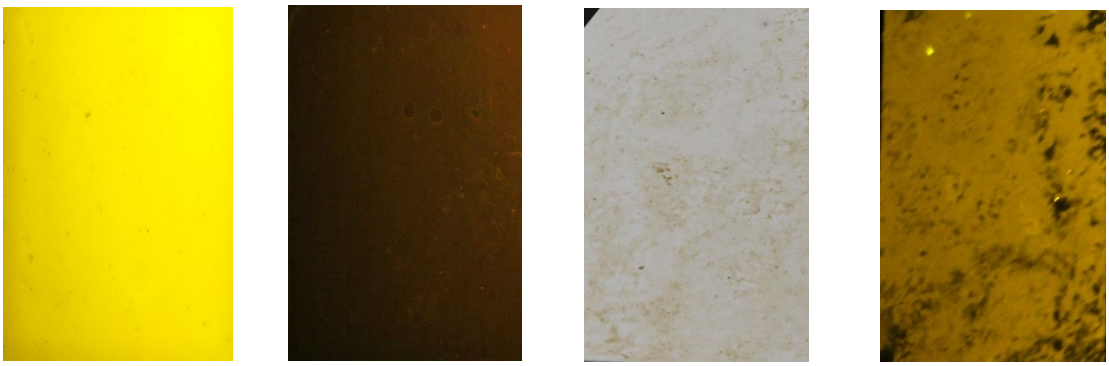
a) Forehead ***b) Body***

Figure 79: Decedent Donor 2 – Day 7 Collection



c) Zar-Pro™ NL ***d) Zar-Pro™ AL***

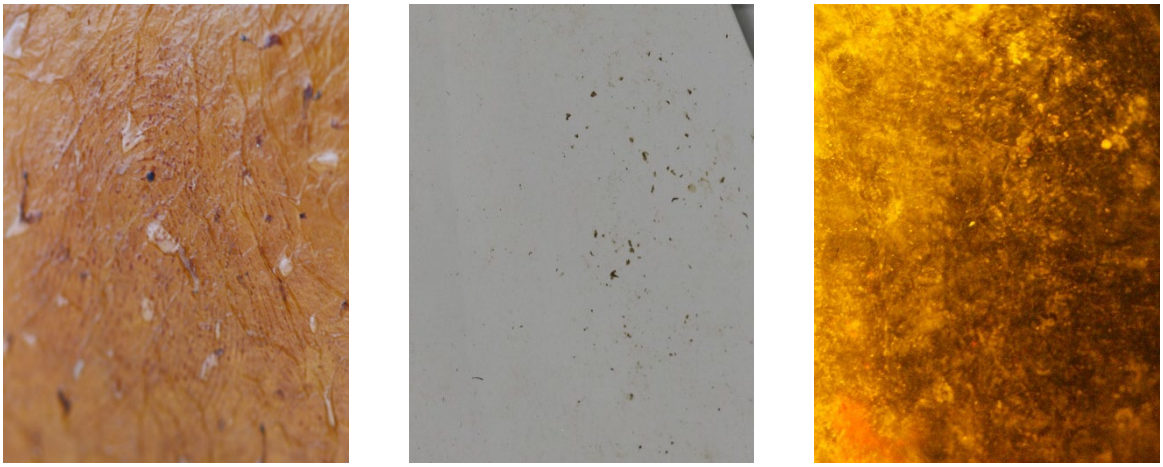
Figure 80: Day 7 Decedent 1- Forehead Non-Impression Controls



a) White Gellifter *b) Black Gellifter* *c) Zar-Pro™ NL* *d) Zar-Pro™ AL*

Figure 81: Day 7 Decedent 2- Forehead Non-Impression Controls

Although faint, blood impressions were still visible insitu on the decedent skin prior to enhancement even on the leg of decedent donor 1 which was more advanced in the stages of decomposition with the epidermal skin no longer present and the dermal skin covered in a greasy residue (Figure 82a). The maggot masses were no longer present, as the protective layer of the epidermis was no longer covering the decedent skin, thus the fly vomit and contamination from the movements were also no longer present as they were primarily focused to the epidermal layer. Zar-Pro™ lifted impressions had proteinaceous materials visible under normal lighting (Figure 82b) but impression details were not visible on the white background of the lifter. When visualized under alternate lighting (82c), the Zar-Pro™ lifted impression was brightly fluorescent but the proteinaceous materials of the greasy dermal layer prevented visualization of any impression details. At this point of decomposition, the Lifters were almost completely contaminated, and a differentiation could not be made from proteinaceous materials from the blood impression versus proteinaceous residue from decomposition.



a) Insitu *b) Zar-Pro™ NL* *c) Zar-Pro™ AL*

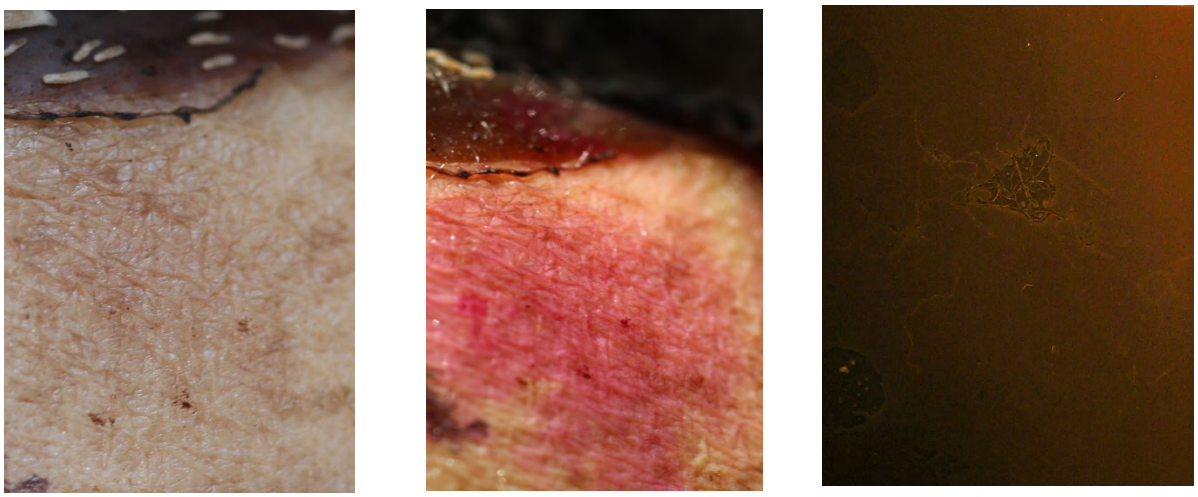
Figure 82: Day 7 Decedent 1- Right Leg Zar-Pro™ Enhanced Impressions

Blood impressions although faint were visible prior to enhancement on the skin of decedent donor 2, most of which looked like blood fingermarks not necessarily blood impressions with the primary distinction being the visibility of impression details (Figures 83a, 84a, and 85a). The decedent still had large maggot masses between the epidermal and dermal layers in areas where the

epidermis was still intact. In some areas, the epidermal and dermal layers were separating over the area of the blood impression (Figure 83a and 85a), revealing the greasy dark layer of the dermal skin. Amido Black dye-stained impressions were visible insitu on the decedents skin (Figure 83b), even with the large maggot mass beneath the epidermal layer. The white Gellifters were not effective at recovering the dye-stained impression, some stain was visible on the Gellifters which also began to destroy the epidermal skin layer when using them on the fragile skin. As the skin was already peeling off the body, the stained area of the epidermis was cut away from the dermal layer (Figure 83c), then cleaned of the maggots (Figure 83d) which allowed for the improved visualization of impression details visible under normal lighting. Hungarian Red dye-stained impressions were not visible insitu with most of the stain absorbing into the dermal layer creating a stained area with no distinction between the stain and the previously existing impression details (Figure 84b). There were some proteinaceous materials visible on the black background of the Gellifters under alternate lighting (Figure 84c), but impression details were not present. Zar-Pro™ lifted impressions had visible impression details on the white background of the lifter under normal lighting (Figure 85b), although they were faint. When visualized under alternate lighting, the impression details of the were brightly fluorescent (Figure 85c), although areas of contamination were also visible. In areas where the epidermis was no longer present, the previous concern of fly contamination was no longer an issue, as the fly contamination was primarily on the epidermal skin layer.

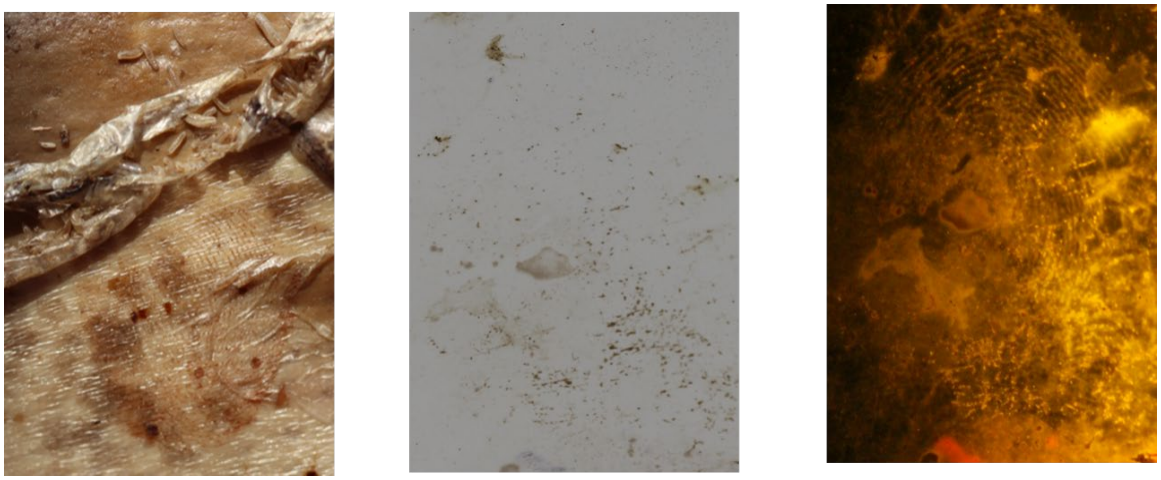
a) Insitu ***b) Insitu Stained*** ***c) Stained Epidermis*** ***d) Stained Epidermis***

Figure 83: Day 7 Decedent 2- Neck Area Amido Black Enhanced Impressions



a) Insitu *b) Insitu Stained* *c) Insitu Stained*

Figure 84: Day 7 Decedent 2- Right Arm Hungarian Red Enhanced Impressions



a) Insitu *b) Zar-Pro™ NL* *c) Zar-Pro™ AL*

Figure 85: Day 7 Decedent 2- Left Leg Zar-Pro™ Enhanced Impressions

Eight-day Collection Interval (B08)

At the eight-day collection interval, maggots and insects were now in large masses which had consumed entire areas of the bodies. The epidermal skin was only present in a few areas on both decedents with the greasy dark colored dermal skin layer present on most of the bodies with some areas even blackening and becoming completely de-fleshed by insect activity (Figures 86 and 87). In areas where the epidermis was still present, blood impressions were detected in situ on the decedent skin (Figure 89a), however the impressions were not as visible on the dermal skin (Figure 88a). Due to the condition of the skin on decedent 1 no more impressions were able to be recovered. The only enhancement method utilized in the later collection intervals were Zar-Pro™ Fluorescent Lifters with impressions to be collected from the upper chest, more specifically the rib margin. The lifted impressions were barely visible under normal lighting (Figures 88b and 89b) with only proteinaceous materials visible on the Lifters. Yet, bright fluorescent impressions were visible on the Lifters under alternate lighting (Figures 89c) when the impression was lifted from the epidermal skin. When the epidermis was gone, only proteinaceous materials in the greasy dermal layer bonded

to the Lifters which were also brightly fluorescent under alternate lighting (Figure 88c), but impression details were not readily visible.

Figure 86: Decedent Donor 1 – Day 8 Collection

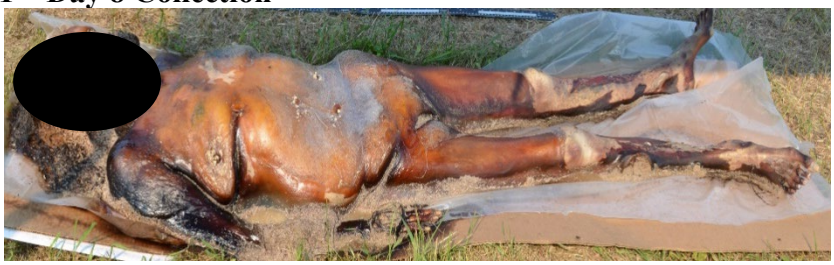
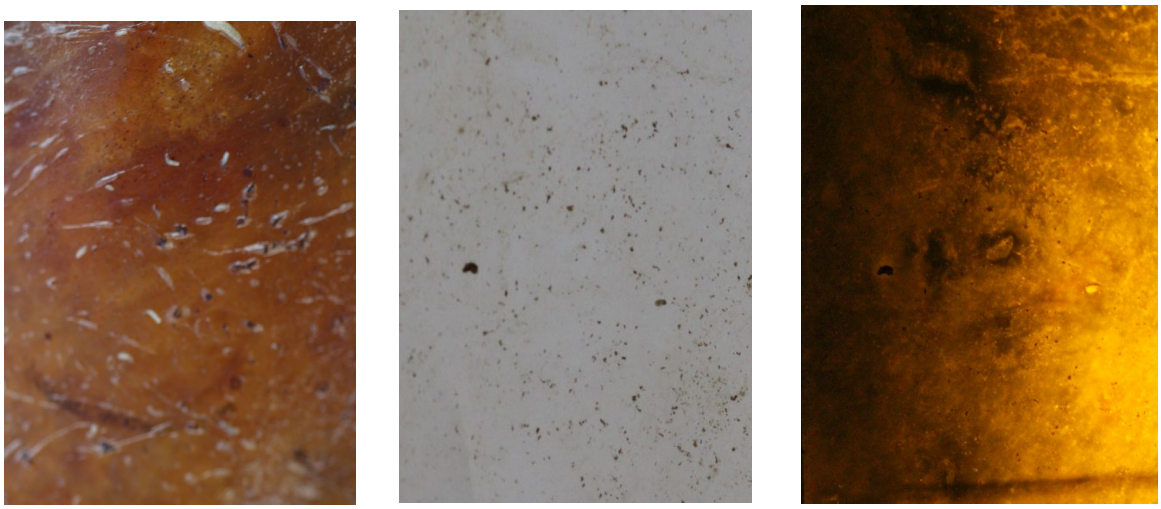
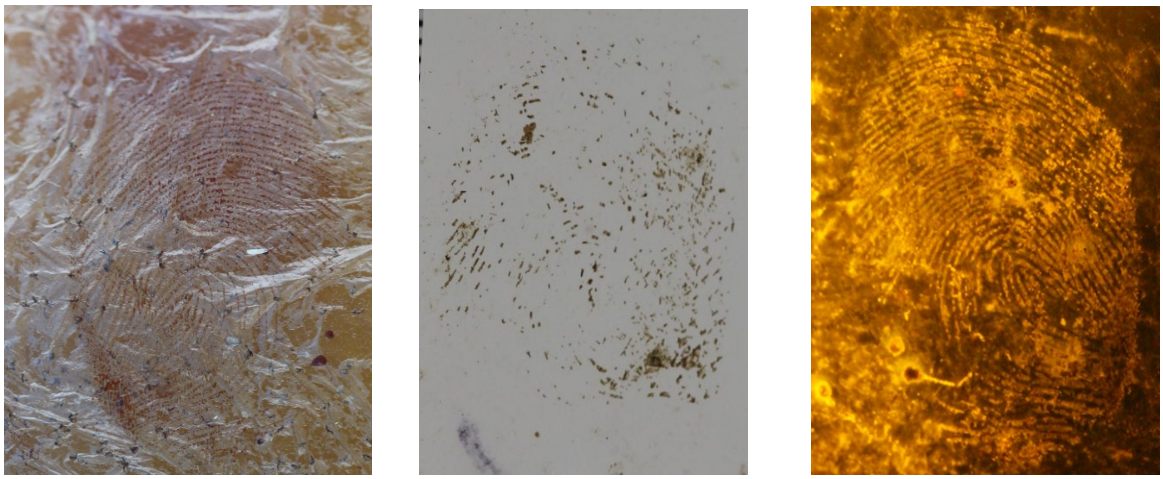


Figure 87: Decedent Donor 2 – Day 8 Collection



a) Insitu ***b) Zar-Pro™ NL*** ***c) Zar-Pro™ AL***

Figure 88: Day 8 Decedent 1 Upper Chest Zar-Pro™ Enhanced Impressions

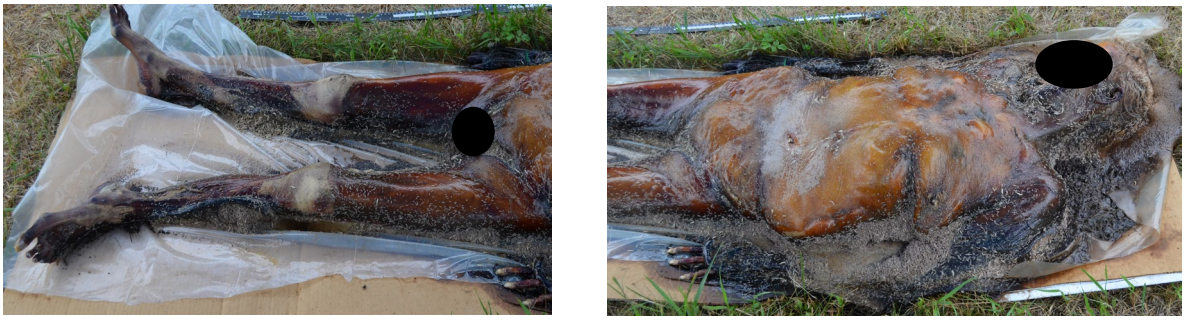


a) Insitu *b) Zar-Pro™ NL* *c) Zar-Pro™ AL*

Figure 89: Day 8 Decedent 2- Upper Chest Zar-Pro™ Enhanced Impressions

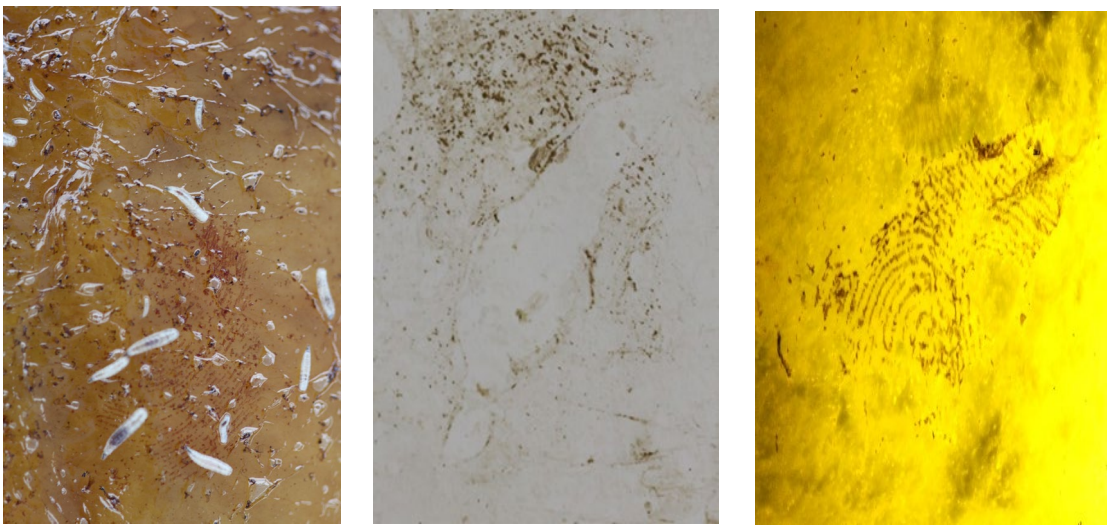
Nine-day Collection Interval (B09)

At the nine-day collection interval, decedent donor 2 was covered only in greasy dark colored dermal skin which was blackening in multiple areas and becoming de-fleshed by insect activity (Figures 90 a and b). Some blood impressions were visible on the dermal skin, meaning the impression deposited ten days earlier absorbed through the epidermis and left a faint outline of the impression onto the dermal layer (Figure 91a), where others were still present on the splitting epidermal skin (Figure 92a). It is interesting to note that even the sharpie outline marking the impression area was shed with the epidermal skin, as it did not appear to penetrate the dermal skin layers. The greasy dermal layer had areas where the skin appeared to be splitting, maggots were still present but not in large numbers as in previous collection intervals. The Zar-Pro™ Fluorescent Lifters were used to recover impressions form the upper chest, more specifically the rib margin and some impression details were lifted. The lifted impressions were barely visible under normal lighting (Figures 91b and 92b) with some impression details (Figure 91c) and even outlines of maggots (Figure 92c) visible on the Lifters under alternate lighting.



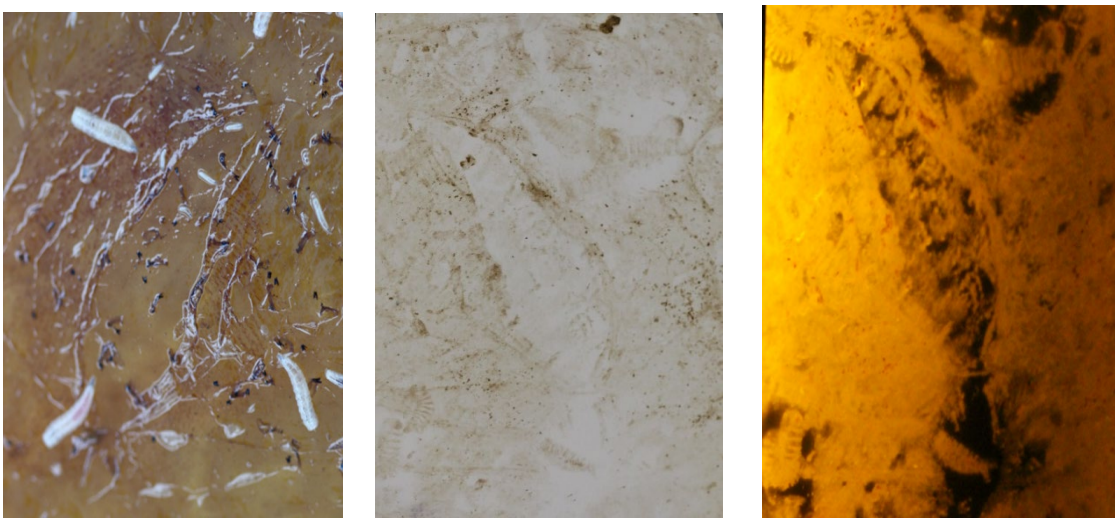
a) Lower Body *b) Upper Body*

Figure 90: Decedent Donor 2 – Day 9 Collection



a) Insitu *b) Zar-Pro™ NL* *c) Zar-Pro™ AL*

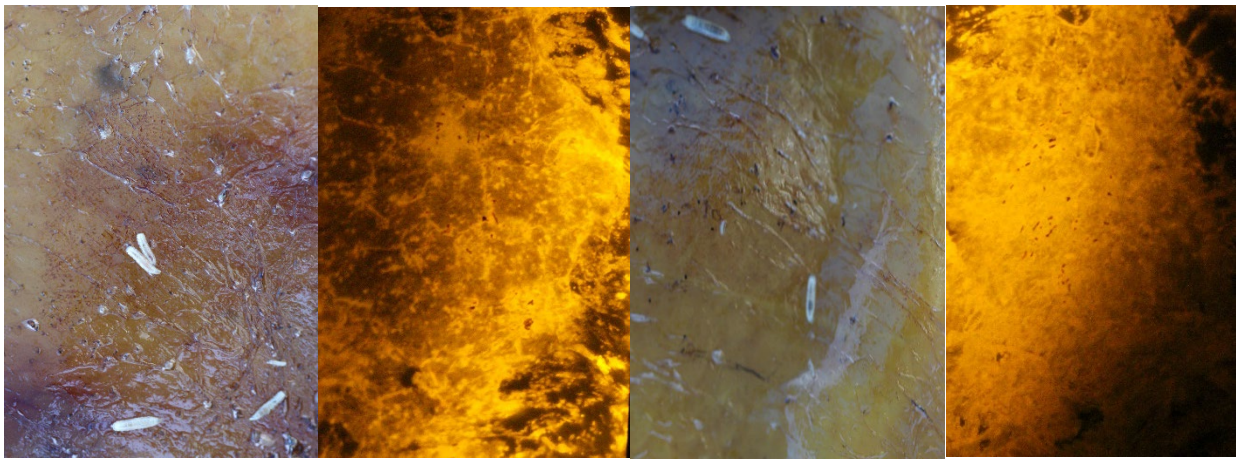
Figure 91: Day 9 Decedent 2- Upper Chest Zar-Pro™ Enhanced Impressions



a) Insitu *b) Zar-Pro™ NL* *c) Zar-Pro™ AL*

Figure 92: Day 9 Decedent 2- Upper Chest Zar-Pro™ Enhanced Impressions

In conclusion, blood can be detected on the dermal skin (Figures 93a and c), but impression details were less likely to be present. Thus, impressions can be recovered with Zar-Pro™ Fluorescent Lifters from decedent skin until the epidermal skin layer was no longer present on the decedent's bodies. At that time, the background contamination from the greasy dermal layer often prevented the visualization of impression details under both normal and alternate lighting (Figures 93b and d). There were some trials that showed the blood impression deposited on the epidermal skin did penetrate the dermal layer and could be visualized in a few instances, but this was relatively rare.

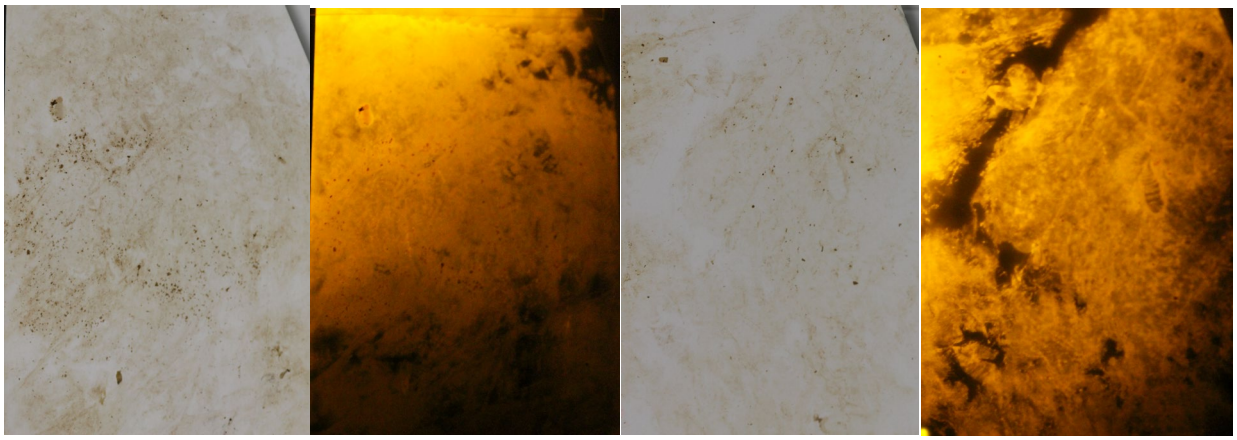


a) Insitu *b) Zar-Pro™ AL* *c) Insitu* *d) Zar-Pro™ AL*

Figure 93: Day 9 Decedent 2- Upper Chest Zar-Pro™ Enhanced Impressions

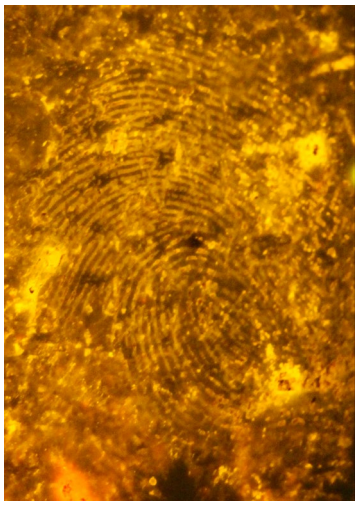
Ten-day Collection Interval (B10)

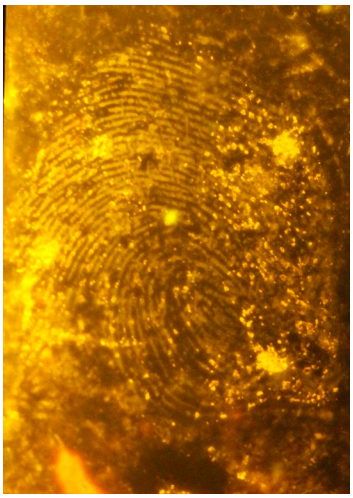
At the ten-day collection interval, the body of decedent donor 2 was almost completely covered in the dark colored greasy dermal skin. The greasy residue along with any residual epidermal skin, prevented the enhancement of any remaining blood impressions. The Zar-Pro™ lifters were only able to recover proteinaceous material, and some maggot outlines from the dermal skin when visualized under normal (Figures 94a and c) and alternate lighting (Figure 94b and d). After this interval, no more impressions were available for recovery. However, there was a fixed blood impression on the upper chest which had been lifted with Zar-Pro™ Lifters on Day 8 (Figure 95a) but was still visible on a small area of epidermal skin that was still intact, so a second Zar-Pro™ lift was applied to the same impression. The lifted impression allowed for the visualization of brightly fluorescent impression details when visualized under alternate lighting (Figure 95b). The second lift collected two days after the original allowed for visualization of impression details that were less contaminated with background fluorescence due to decomposition and/or insect activity. At the later collection intervals maggots were also squished and lifted onto the Lifters (Figure 94a and c) which could account for additional background contamination.



a) Zar-Pro™ NL *b) Zar-Pro™ AL* *c) Zar-Pro™ NL* *d) Zar-Pro™ AL*

Figure 94: Day 10 Decedent 2- Upper Chest Zar-Pro™ Enhanced Impressions





a) First Lift Day 8 *b) Second Lift Day 10*

Figure 95: Day 10 Decedent 2- Upper Chest Zar-Pro™ Enhanced Impressions

9. Analysis of Examiner Ratings

Four data sets were compiled from the archived digital images as follows: Data Set 1included the one-hour control impressions (BC1 and NC1) and the four-day (B04) collection interval, Data Set 2 included the one-day (B01) and two-day (B02) collection intervals, Data Set 3 included the three-day (B03) and six-day (B06) collection intervals, and Data Set 4 included the five-day (B05), seven-day (B07), eight-day (B08), nine-day (B09), and ten-day (B10) collection intervals. Data sets1-4 were generated to access inter-examiner variability, whereas Data Set 5 was generated to assess intra-examiner variability. Thus, Data Set 5 consisted of impressions that had both agreement and variability amongst examiners from the previously reported data sets. Data sets were compiled based on the number of digital images associated with each set, the earlier days allowed for the recovery of more impressions thus were larger than the data sets collected as decomposition progressed. Each Data Set had an associated rating sheet for the examiners to complete.

The Data Sets and associated rating sheets for the human decedent trials were organized (Data Sets 1-5), archived, and then posted to Dropbox for access by two student examiners and two practicing latent print analysts, which can be accessed via the following links:

Data Set 1:
https://www.dropbox.com/sh/o09wp7fj9s4n27x/AABZufPJ9uegjxyW3bkWtQmJa?dl=0

Data Set 2:
https://www.dropbox.com/scl/fo/xeqbmsxi40bktl4h59dj1/h?dl=0&rlkey=w9dhr5qb0kf4kg0a9pskxo6wd

Data Set 3:
https://www.dropbox.com/scl/fo/mpxb1maplajk3dmrvcm0z/h?dl=0&rlkey=mt1jvpjh1826okt594tt7sq89

Data Set 4:
https://www.dropbox.com/sh/ppslrsrcrrmgm34/AACaAwfQEeI_h-RsDjWLW1Tka?dl=0

Data Set 5:
https://www.dropbox.com/scl/fo/9ebx7klhymaynqj9u9sxf/h?rlkey=1hd8cihlevae81pefy5zhpgf3&dl=0

Examiner Rating Sheets:
https://www.dropbox.com/scl/fo/62siqnfut5xk7mqd9gz98/h?rlkey=68bx1rxovdql8p0na6gagctk7&dl=0

Examiner Ratings of Impression Details (ID) and Fluorescent Intensity (FI)

The two student examiners and two practicing latent print analysts rated the recovered blood impressions from the skin of decedent donors 1 and 2 in the human decedent trials. The examiners rated the Impression Details (ID) ranging from 0-4 and Fluorescent Intensity (FI) ranging from 0-6 using the provided scale standards (Figures 4 and 5). The impressions were rated from digital images of the blood impressions insitu on the decent skin prior to enhancement and insitu on the decedent skin after being dye-stained with Amido Black and Hungarian Red under normal lighting conditions. Ratings were also conducted from the digital images of the recovered impressions that were lifted from the decedent skin. White BVDA Gellifters® were utilized to lift the Amido Black stained impressions and were only photographed under normal lighting conditions as Amido Black is not fluorescent. Black BVDA Gellifters® were utilized to lift the Hungarian Red stained impressions which were photographed under both normal and alternate lighting, as Hungarian Red is fluorescent. Zar-Pro™ Fluorescent Lifters were also utilized to recover the blood impressions and were photographed under normal and alternate lighting. The recovered impressions, meaning the impressions lifted with the Gellifters and Zar-Pro™ Lifters were photographed one hour after each collection interval and then photographed again one-month later.

The impression details and fluorescent intensity ratings were amalgamated and averaged for each of the three enhancement methods between the two student examiners and between the two-practicing latent print analysts. The average ratings between the two groups were kept separate to provide a rating assessment based on education, training, and experience amongst examiners. One of the practicing latent print analyst, Examiner B chose not to rate impression details in 140 of the 1,283 digital images due to image quality, which were described as out of focus. Of the impressions that were not rated 62% of those digital images were of the impression area on the decedent skin prior to enhancement, 30% of the digital images were of the Amido Black or Hungarian Red stained impressions insitu on the decedent skin and only 8% were from the lifted impressions. This was a foreseen limitation of the study, as the insitu photography on decedent skin is more difficult than the photography of lifted impressions affixed to the BVDA Gellifters® and Zar-Pro™ Lifters which were photographed in the controlled setting of the laboratory. The fluorescent intensity ratings from digital images taken under alternate lighting were not affected by the clarity of the images as alternate light photography was also conducted in the laboratory.

Amido Black Ratings Assessment

Blood impressions were visualized insitu on the decedents skin prior to enhancement for each collection interval, both the students and analyst rated the impressions prior to enhancement for D1 between a 1.33 rating and 2.5, meaning proteinaceous material and some ridge details were visible (Appendix E; Graph 1) through the four-day collection interval. The ratings for D2 were slightly higher than D1 through the four-day collection interval with a low rating of 1.75 and a high rating of 2.92, meaning proteinaceous partial to full impression details were visible prior to enhancement (Appendix E; Graph 5). For both decedents the ratings decreased at the five-day collection interval and continued to decrease through the seven-day collection interval. The day five ratings ranged from 0.59-1.84 for both decedents, meaning proteinaceous material was visible but the impression details were no longer as readily visible. At the six-day collection interval D1 had a high rating of 0.09, meaning proteinaceous materials were not visible, whereas D2 had a low rating of 1.54 and a

high rating 1.83 thus some partial impression details were still visible. Due to the condition of D1 no impressions were recovered at the seven-day collection interval but D2 had ratings ranging from 0.65-1.3 at this interval, meaning proteinaceous materials were still visible (Appendix E; Graphs 1 and 5).

The insitu staining on the skin of the decedents using Amido Black dye stain increased the visualization of impression details in every rating from the pre-enhancement comparison (Appendix E; Graphs 1, 2, 5, and 6). Impression ratings for D1 ranged from a 1.67 to a 3 through the five-day collection interval, meaning proteinaceous partial to full impression details were visible after application of the dye-stain. At the six-day collection interval for D1, the impression ratings dropped from a high of 2.84 at the five-day interval to a low rating of 0.25 meaning proteinaceous materials were no longer readily visible (Appendix E; Graph 2). The impression ratings for D2 were higher than the ratings for D1 and ranged from a 2 to 3 through the five-day collection interval, meaning after the application of the dye-stain partial to full proteinaceous impression details were readily visible. At the six and seven-day collection interval for D2 the ratings ranged from 1.4 to 2.34, meaning proteinaceous material and some ridge details were still visible, thus D2 did not lose impression details after the five-day interval like D1. (Appendix E; Graph 6).

Using the white BVDA Gellifters® to lift the Amido Black stained impressions reduced the impression detail ratings from the insitu enhancement in every collection interval for D1 and D2 with one exception, the student examiners rated the Gellifters from D1 at the one-hour collection interval a 2.67 which they had rated a 2.17 for the dye-stained insitu enhancement. The analyst rating at this interval however did decrease from a 3 for the insitu enhancement to a 2.84 on the Gellifter, both of which were high ratings meaning partial to full impression details were visible on the Gellifters (Appendix E; Graphs 2, 3, 6, and 7). From the day-one collection interval through the seven-day collection interval, the effectiveness of the Gellifters varied in effectiveness with D1 ranging from 0.34 to 2.5 which were better than D2 which ranged from 0.17 to 1.84 (Appendix E; Graphs 3 and 7). When the Gellifters were rated again one month after the trial was completed, the impression details ratings either stayed the same or decreased slightly (Appendix E; Graphs 4 and 8). Thus, the Amido Black dye-stained impressions are still present on the Gellifters after recovery and can be assessed one month later.

Hungarian Red Ratings Assessment

Blood impressions insitu on the decedents skin prior to Hungarian Red dye-stained enhancement had a low rating of 0.42 at the six-day collection interval with a high rating of 2.42 at both the four and five-day interval for D1 (Appendix E; Graph 9). The pre-enhancement ratings for D2 ranged from a low of 0 at the six and seven-day collection intervals to a high of 3 at the one-hour interval (Appendix E; Graph 15) with D2 having higher average ratings than D1 through the five-day interval but lower rating in the six and seven-day intervals (Appendix E; Graphs 9 and 15). In the assessment of the impression rating prior to enhancement there was no noticeable trend detected, and the ratings went from a 0 to 3 which is the full spectrum of the impression detail rating standard meaning some impressions had no visible proteinaceous materials whereas other impressions had full impression details with ridge path deviations visible.

The insitu staining on the skin of the decedents using Hungarian Red dye stain either stayed the same or increased the visualization of impression details for most of the impression rating from the pre-enhancement comparison (Appendix E; Graphs 9, 10, 15 and 16). Impression ratings for D1 ranged from a 1.6 to a 2.82 through the five-day collection interval, meaning after the application of the dye-stain proteinaceous materials were visible to some partial to full impression details were

visible. At the six-day collection interval for D1, the impression ratings dropped from a high of 2.42 at the five-day interval to a low rating of 0.34 meaning proteinaceous materials were no longer readily visible (Appendix E; Graph 10). The impression ratings for D2 were higher than the ratings for D1 and ranged from a 2.33 to a 3 through the five-day collection interval, meaning after the application of the dye-stain partial to full proteinaceous impression details were visible. At the six-day collection interval for D2, the impression ratings dropped from a high of 3 at the five-day interval to a low rating of 0 meaning proteinaceous materials were no longer readily visible at the six or seven-day interval (Appendix E; Graph 16).

Using the black BVDA Gellifters® to lift the Hungarian Red stained impressions significantly reduced the impression detail ratings from the insitu enhancement in every collection interval for D1 and D2 (Appendix E; Graphs 10,11, 16 and 17). Impression details at the one-day collection interval for D1 had the highest rating of 2.82 and was reduced to a 0 rating on the Gellifters (Appendix E; Graphs 10 and 11). This was also seen on D2 with the two-, three-, and four-day collection intervals sharing a high rating of 2.84 which was again reduced to a 0 rating on the Gellifters (Appendix E; Graphs 16 and 17). The decedents had a range of ratings from 0 to 1 for all the collection intervals, meaning no proteinaceous material was visible to proteinaceous materials may be visible but impression details were not visible. When the Gellifters were rated again one month after the trial was completed, the impression detail ratings tended to decrease or stay the same (Appendix E; Graphs 13 and 17). Thus, just like the Amido Black dye-stained impressions, Hungarian Red dye-stain impressions were still present on the Gellifters one month after recovery, but the effectiveness of the lifter is lacking.

Due to the fluorogenic properties of Hungarian Red, the lifted dye-stained impressions were visualized on the Gellifters under alternate lighting, which was in addition to the ratings under normal lighting. The Gellifters were rated for both impression detail and the fluorescent intensity under the alternate lighting which appeared to only slightly improved the visualization proteinaceous materials which were not visible on the lifter under normal lighting (Appendix E; Graphs 12, 14, 18, and 20). The decedents had a low rating of 0 with a high rating of 1 through the seven-day collection intervals meaning the best ratings were only able to detect the presence of proteinaceous materials on the Gellifters under alternate lighting. This was primarily due to the fluorogenic properties of Hungarian red which allowed for the visualization of fluorescence which ranged in fluorescent intensity from 0 to 3.5 on the provided fluorescence scale standard (Appendix E; Graphs 12 and 18). When the Gellifters were rated again one month after the trial was completed under alternate lighting, the impression detail ratings increased slightly again ranging from 0 to 1, with the fluorescent intensity increasing to a range of 0 to 5.17 (Appendix E; Graphs 14 and 20). Although the fluorescent intensity increased, the overall impression detail rating did not surpass a 1, meaning the fluorescence did not allow for the visualization of any impression details.

Zar-Pro™ Fluorescent Lifters Ratings Assessment

Blood impressions insitu on the decedents skin prior to Zar-Pro™ enhancement had a low rating of 0 through the eight-day collection interval with a high rating of 3 at one-hour interval for both D1 and D2 (Appendix E; Graphs 21 and 26). As with the other enhancement methods Zar-Pro™ Lifters were used through the eight-day collection interval for both D1 and D2, however additional blood impressions were recovered from D2 through the ten-day collection interval. The ratings for the nine and ten-day collection intervals had a low rating of 1.25 and a high rating of 1.67, meaning proteinaceous materials with some ridge details were visible (Appendix E; Graph 26). As with the other impression ratings prior to enhancement, the impression ratings were variable amongst the

collection intervals meaning some impressions had no visible proteinaceous materials whereas other impressions had full impression details with ridge path deviations.

Blood impressions were visible under normal lighting on the Zar-Pro™ Lifters with a low rating of 2.83 and a high rating of 3 for both D1 and D2 through the three-day collection interval (Appendix E; Graphs 22 and 27), meaning the overall impression pattern with ridge details were readily visible. The ratings on the Zar-Pro™ Lifters increased the visibility of impression details from the pre-enhancement ratings except for the two ratings that were previously rated a 3 at the one-hour intervals. However, between the four- and eight-day intervals the impression details generally decreased under normal lighting with a high of 2.59 and a low rating of 0 for both D1 and D2. Through the ten-day collection interval D2 had a low rating of 0 and a high of 1.33 (Appendix D: Graphs 22 and 27). The range in impression details from the four to ten-day collection intervals mean no proteinaceous materials were visible to some proteinaceous impressions were visible with ridge details during these intervals. The lower ratings are primarily due to the loss of the reddish coloration of blood which was no longer readily visible on the white background of the Lifters under normal lighting. These ratings generally did not change after recovery between the one hour to a month post enhancement rating under normal lighting (Appendix E; Graphs 22, 24, 27, and 29).

Due to the fluorogenic properties of the Zar-Pro™ Lifters proteinaceous materials can be fluoresced under alternate lighting which can increase the visibility of impression details when the Lifters are moisture-free. The images taken under alternate lighting one hour after recovery were not as brightly fluorescent as the images taken one month later due to the moisture from the Zar-Pro™ activator quenching the Lifters fluorescence. When the Lifters were visualized under alternate lighting through the three-day collection interval, the Lifters again had a low impression detail rating of 2.83 and a high of 3 for D1 meaning the overall impression pattern with ridge details were readily visible with fluorescence ranging from 2.42-3.34 (Appendix E; Graphs 23). One month after recovery the impression detail ratings through the three-day interval remained the same for D1 but the fluorescent intensity increased now ranging from 4.83-6 which is the highest fluorescent intensity on the provided scale (Appendix E; Graph 25). A similar increase in fluorescence was seen for D1 on the four to eight-day collection intervals with the impression detail ratings having a high of 2.59 and a low of 0 with fluorescent intensity ranging from 0.5-4.92 one hour after recovery (Appendix E; Graph 23). One month later, the impression detail ratings increased to a high of 2.92 with fluorescent intensity increasing to a range of 1.5-6 (Appendix E; Graph 25), meaning the increase of fluorescence improved the visibility of impression details. This phenomenon was also seen in the D2 ratings, one hour after recovery the impression details were rated a low of 2.09 and a high of 3 with fluorescent intensity ranging from 2-3.67 through the three-day collection interval (Appendix E; Graph 28). One month after recovery, impression details rating had a low of 2.75 with fluorescent intensity now increasing to a high of 5.25 through the three-day collection interval (Appendix E; Graph 30), again showing the impression detail ratings improved as fluorescence intensity increased. In the four-to-eight-day intervals, impression detail ratings had a high of 3 and a low of 0.45 with fluorescent intensity ranging from 1.09-5 one hour after recovery (Appendix E; Graph 28). One month later in the four to eight-day collection intervals impression details increased to a low of 0.67 with fluorescence intensity increasing to a high 5.67 (Appendix E; Graph 30). At the nine and ten-day collection intervals for D2, the impression detail rating did not change between the one hour and one month ratings after recovery with a low of 0 and a high of 1.33, although fluorescent intensity increased from 1-5 to the range of 2.34-6 (Appendix E; Graphs 28 and 30), the increase in fluorescence did not improve the visualization of impression details lifted from decedent skin during the later stages of decomposition

Statistical Assessment

Cohen's Kappa statistical analysis was calculated for the inter-examiner variation between the two student examiners and two practicing latent print analysts for Data sets 1-4. The student examiners' ratings were almost perfect (1) for impression details under both normal and alternate lighting. However, the strength of agreement decreased for fluorescent intensity to a range of moderate (0.53) to substantial (0.75) (Appendix F; Table 1). A similar assessment was observed in the ratings of the practicing latent print analysts with almost perfect agreement (0.91-1) for impression details under both normal and alternate lighting. The strength of agreement decreased slightly for fluorescent intensity ranging from moderate (0.51) to almost perfect (1) (Appendix F; Table 2). The ratings from Latent Print Analyst A were compared to Student 1 with again an almost perfect (0.89-1) level of agreement for impression detail under both normal and alternate lighting and a moderate (0.41) to substantial (0.72) level of agreement for fluorescent intensity (Appendix F; Table 3). The level of agreement was similar for impression details with an almost perfect (0.95-1) agreement under both normal and alternate lighting for Latent Print Analyst A to Student 2. However, the level of agreement amongst them for fluorescent intensity dropped to poor (-0.5) to substantial (0.61), which was a drastic decrease in rating agreement (Appendix F; Table 3). The same assessment was conducted for Latent Print Analyst B to Student 1 with an almost perfect (0.87-1) level of agreement for impression detail under both normal and alternate lighting and a slight (0.19) to almost perfect (0.82) level of agreement for fluorescent intensity (Appendix F; Table 4). The level of agreement was similar for impression details with an almost perfect (0.89-1) agreement under both normal and alternate lighting for Latent Print Analyst B to Student 2. However, the level of agreement amongst them for fluorescent intensity dropped to poor (-0.8) to moderate (0.43) (Appendix F; Table 4). As observed in the previous rating assessment between Analyst A and Student 2 the same decrease in fluorescence intensity was seen between Analyst B and Student 2.

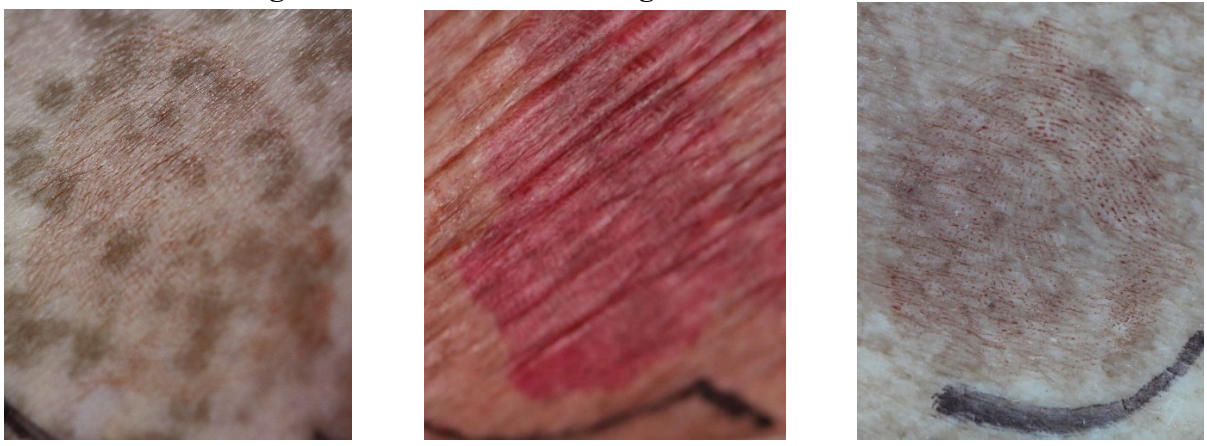
Data Set 5 was an assessment of intra-examiner ratings, meaning the data set was compiled from previous rated digital images thus allowing for the comparison of the current rating to the previous rating provided for the same image. Another inter-examiner assessment was also conducted from the intra-examiner ratings. The inter-examiner assessment for all examiners was almost perfect (0.97-1) for impression details under both normal and alternate lighting but ranged from slight (0.05) to substantial (0.76) for fluorescent intensity (Appendix F; Graph 5). Latent Print Analyst A had the highest level of agreement amongst the examiners with an almost perfect level of agreement (1) for impression details under both normal and alternate lighting, meaning there was minimal variation between the ratings provided for the same image. The fluorescent intensity ratings were also the highest with almost perfect (0.82-0.92) agreement of their ratings for fluorescent intensity. Student 1 also had almost perfect (0.95-1) level of agreement for impression details under both normal and alternate lighting and almost perfect (0.82-0.91) agreement for fluorescent intensity. Student 2 also had an almost perfect (1) level of agreement for impression details under both normal and alternate lighting but only slight (0.17) to moderate (0.51) agreement for fluorescence intensity. Latent Print Analyst B had the lowest level of agreements yet was still in the almost perfect (0.85-0.97) range for impression details under both normal and alternate lighting and only had substantial agreement (0.61-0.82) for fluorescent intensity (Appendix F; Table 5).

The variation seen in the intra-examiner impression detail ratings for Latent Print Analyst B was likely due to the examiner's inconsistencies in impression ratings. The Examiner did not rate some of the digital images as they were deemed out of focus (no rating provided) in the inter-examiner analysis of data sets 1-4 but the same digital images were later rated with a 3 for impression details

in the intra-examiner analysis of data set 5 (Figures 96a, c and 97c). The images were from both decedent donors 1 and 2 at the one- (B01) and three-day (B03) collection intervals, two images were insitu pre-enhancement for Zar-Pro ™ (EC) from the right leg (LRL) and neck (LNK) under normal lighting (NL) (Figures 96a and 97c). The other image changed from out of focus (no rating provided) to a 3 in the intra-examiner ratings was from decedent donor 1 at the one-day collection interval from a Zar-Pro™ Lifter photographed one-hour after collection under alternate lighting (AL) (Figure 96c). In contrast, some of Examiner B original impression detail ratings were 1 and 2, which were changed to out of focus (no ratings provided) in the intra-examiner data set (Figure 96b, 97a and b). The images were from both decedent donors 1 and 2 at the one-hour (BC1) interval, one image was insitu pre-enhancement for Zar-Pro™ (EC) from the left leg (LLL) under normal lighting (NL) which was originally rated a 2 then changed to out of focus (no rating provided) in the intra-examiner analysis (Figure 97a). The other two images were insitu on the decedent skin after dye-staining with Amido Black (EA) (Figure 96b) and Hungarian Red (EB) (Figure 97b). The Amido black stained impression was originally rated a 2 and the Hungarian Red stained impression was rated a 1, both of which were changed to out of focus (no rating provided) in the intra-examiner analysis.

a) EC-LRL-B01-NL ***b) EB-LRA-BC1-NL*** ***c) EC-LNK-B03-AL***

Figure 96: Examiner B Rating Variations- Decedent 1



a) EC-LLL-BC1-NL ***b) EB-LRA-BC1-NL*** ***c) EC-LNK-B01-NL***

Figure 97: Examiner B Rating Variations – Decedent 2

Significant Results

Blood impressions or at least proteinaceous fingermarks were able to be visualized on the decedent skin through most of the collection intervals even through the later stages of early decomposition (7-10 days) if the epidermal skin was still present. The use of the dye-stains Amido Black and Hungarian Red to enhance blood impressions insitu on decedent skin allowed for improved visualization of impression details according to the rating assessments. Thus, the impression quality increased from the rating of the impressions prior to and post enhancement. Amido Black was effective as an insitu dye stain through the six to seven-day collection intervals, whereas Hungarian Red was effective as an insitu dye stain primarily through the five-day collection interval, depending on the level of decomposition in the body area. The use of the white and black Gellifters to lift the dye-stained impressions from the decedent skin were not effective as most of the impression details were lost in transfer with the rating assessments decreasing in impression quality from the insitu stained impressions. The Hungarian Red dye-stained proteinaceous materials on the black Gellifters were fluorescent when visualized under alternate lighting ranging in fluorescent intensity from a high of 3.5 when observed one hour after recovery to a high of 5.17 out of 6 on the scale standard one month later. Although the increase in fluoresce was observed, likely due to the reduction of moisture within the stain affixed to the Gellifters which can quench fluorescence, the marked increase in fluorescence intensity dd not improve the visualization of impression details. The Zar-Pro™ Fluorescent Lifters were effective in lifting and enhancing blood impressions from decedent skin through the ten-day collection interval, in part due to their fluorogenic properties. Lifted blood impressions were readily visible on the white background of the Lifters through the three and four-day collection intervals, at which time the impression details were becoming faint and not readily visible under normal lighting. However due to their fluorescent properties' impression details were visible on the Zar-Pro™ Lifters through eight-days with a double lift collected at the ten-day collection interval. The fluorescent properties of the Lifters are optimized once free of moisture thus the fluorescent intensity at one hour range had a high of 4.92 which increased to a high of 6, the highest rating on the scale standard one month later. Unlike Hungarian Red, the fluorescent properties of the Zar-Pro™ lifted impressions translated to higher impression detail assessments when visualized under alternate lighting. The ability to lift the blood impression from the skin substrate also allowed for improved visualization of impression details, which was a limiting factor when using the insitu dye-stains. The limiting factor for the effectiveness of the Lifters was the presence of the epidermal skin, if the epidermal skin was intact the impression could be recovered, albeit biofluid contamination did hinder the visualization of some impression details. During the advanced stages of early decomposition when only the greasy epidermal skin remained, the Lifters recovered too much biofluid contamination that even though the lifts were brightly fluorescent impression details could no longer be visualized.

All three enhancement methods allowed for the improved visibility of blood impressions on decedent skin with overall impression quality decreasing through the ten-day trial period. This was expected as the breakdown of the skin and artifacts associated with decomposition would naturally affect the recoverability of blood impression from the skin of human decedents during the early stages of decomposition. Cohens Kappa statistical assessment for examiner ratings determined almost perfect agreement for impression detail ratings under both normal and alternate light. This level of agreement was seen in the inter-examiner assessments of student to student, analyst to analyst, analysts to students, as well as in the intra-examiner assessments. Concluding the examiners had significant agreement in their rating of impression details through the human decedent trials. This level of agreement was not seen in the inter-examiner assessment of

fluorescent intensity with ranged from moderate to almost perfect agreement amongst the analyst but only moderate to substantial agreement amongst the students. In comparing the analysts to the students, the level of agreement on fluorescent intensity ranged from poor to almost perfect. This level of disagreement was also seen in the intra-examiner assessments ranging from slight to almost perfect depending on the individual examiner ratings. The variation in ratings for fluorescent intensity concludes examiners were not in agreement with fluorescent intensity using the provided scale standard. However, it was interesting to note, the analysts and students were most in agreement amongst each other (analyst to analyst and student to student) regarding the fluorescent intensity ratings. Due to the variation, when providing fluorescent intensity in the trials, a range of fluorescent intensity was reported which reflects the ratings of all examiners.

Detection and Visualization of Blood and Semen on Skin using Alternate Lighting

At the six-day collection interval, blood impressions were beginning to be more difficult to detect under normal lighting. To study the use of alternate lighting to help detect blood and other biological fluids on decedent skin the portable dark room tent (Figure 3) was set up atop the decedent donors to allow for the use of the alternate light source to visualize the skin of the decedents. The handheld Rofin® Polilight Flare Plus II with the 450, 505, and 545nm wavelengths and yellow, orange, and red barrier filters were used to assess the value of the light source for application in forensic cases.

Alternate Lighting 450nm

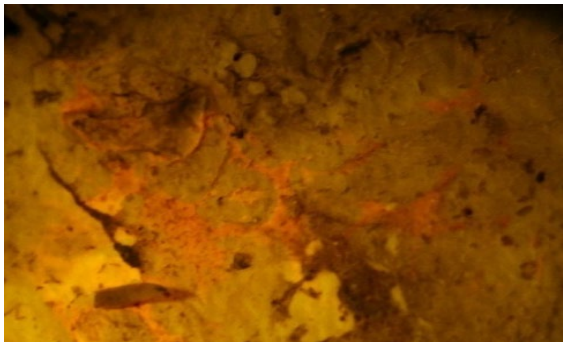
The 450nm wavelength with yellow barrier filter allowed for the visualization of darkened impression areas of unenhanced blood impressions on the bluish-black hue of the decedent skin. Some blue fluorescence was observed of the maggots, but the Hungarian Red dye stain was not fluorescent. The Amido Black stained impressions were darkened allowing for visibility of ridge details. The orange barrier filter allowed for the darkening of unenhanced ridge details, the Amido Black stained impressions were again darkened, and the Hungarian Red stained impressions were weakly fluorescent. The dermal layer appeared darker than the epidermal layer, proving a contrast for visualization of impressions on the epidermal skin. The red barrier filter did not improve the contrast for visualization of impression details. The blood impressions were only slightly more visible than under normal lighting. Amido Black stained impressions were slightly darkened, and Hungarian Red stained impressions were not fluorescent or readily visible.

Alternate Lighting 505nm

The 505nm wavelength with the yellow barrier filter was very bright but did not necessarily improve the visualization of impression details which were slightly darkened. The separation between the epidermal and dermal skin was visible but there was no fluorescence observed from the biofluids or from the Hungarian Red stained impression area. Amido Black impressions were darkened slightly but the brightness was distracting from impression visualization. The orange barrier filter produced superior results, so the skin condition was documented using the Cannon EOS T5i digital SLR camera with the EF 100mm Macro lens. Blood on the decedent skin darkened but so did the blood and biofluids transported over the skin through insect activity, primarily from fly and maggot movements (Figure 98a and b), which obscured some of the impression details. The epidermal skin was weakly fluorescent, but the underlying dermal skin was more brightly fluorescent (Figures 98a, b, and c), this can be seen where the epidermal and dermal skin were splitting (Figure 98b), and over open wounds (Figure 98d). The maggots were also darkening on the

epidermal skin (Figure 98c) with the continual movements distracting when attempting to visualization blood impressions. The Amido Black stained impressions were darkened but the background fluorescence did not necessarily improve visualization of impression details than when observed under normal lighting. Hungarian Red stained impressions were brightly fluorescent (Figure 99a), but the impression details were obscured due to excess background staining in the impression area (Figure 99b). Semen smears were also brightly fluorescent and were readily visible on the epidermal skin (Figure 99e). Sharpie marks outlining the impression areas also darkened and were readily visible (Figure 99 d and e). The red barrier filter produced weakly fluorescent Hungarian Red stained impressions, and a fluorescent epidermal layer with some darkening of blood but the results were inferior to the orange barrier filter.

a)Dark Staining Biofluids- Fly Contamination



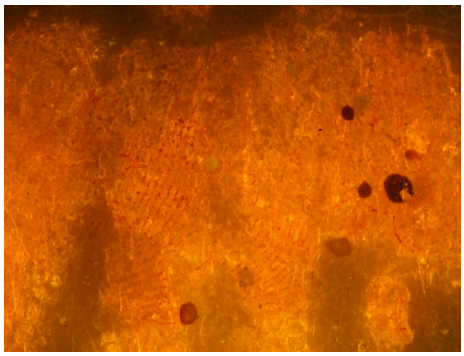
b) Skin Splitting- Epidermal/Dermal Layers

c) Darkening of Maggotts

d) Fluorescene of Dermal Skin

e) Fluorescen of Semen Smear

Figure 98: Alternate Light Photography of Decdent Skin- Day 6



a) Insitu Stained Impression ***b)Background Staining***

Figure 99: Hungarian Red Dye-Stained Impressions Visualized Insitu on Skin

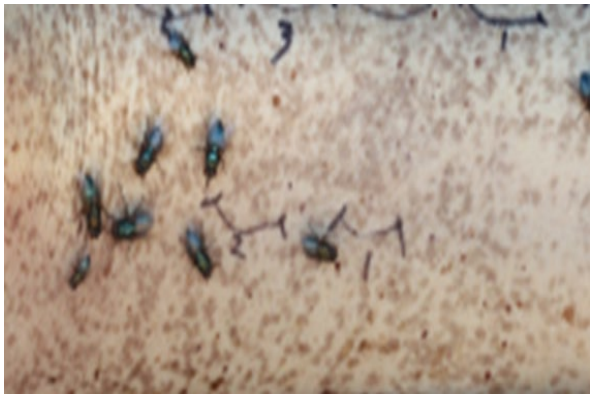
Alternate Lighting 545nm

The 545nm wavelength with the yellow barrier filter was very bright, creating less separation of the darkening blood on the fluorescent epidermal skin layer. The orange barrier filter allowed for visualization of darkened unenhanced and Amido Black stained impressions, but Hungarian Red was only weakly fluorescent. The red barrier filter did not allow for a great contrast of the unenhanced blood impressions, but Amido Black stained impressions were darkened and visible. Hungarian Red was also not fluorescent with this barrier filter at the 545nm wavelength.

Improving Visualization by Lifting Impressions to Remove Substrate Variables

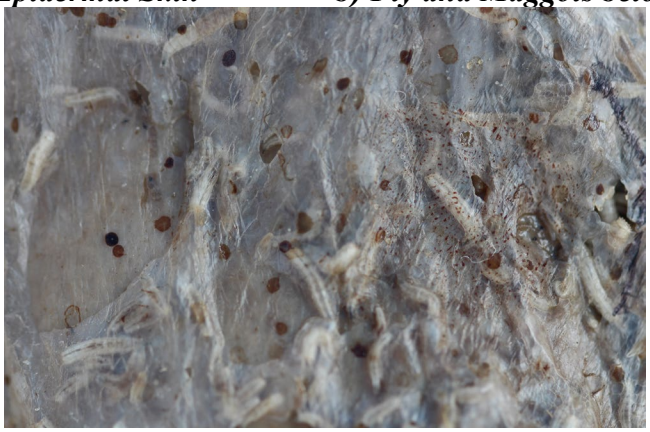
Neither of the decedent donors had significant dermal pattern that affected the visualization of the blood impressions on their skin, as both had light-colored skin with some freckling, moles, and liver spots, but neither were overly hairy. These dermal artifacts, many of which can mimic the fine details present in impressions can obstruct the visualization of impression evidence when visualized insitu on the decedents skin. The substrate variables on the decedent skin during the early collection intervals that were more limiting were skin porosity, body contours and curvatures, skin creases, as well as dermal textures such as mold spots, skin peeling and skin slippage which were seen as early as the one-hour collection interval. As neither decedent had significant substrate variables associated with their skin, the artifacts of decomposition were more pertinent to the visualization of impression details during the early stages of decomposition. During the first one to three-days of decomposition, flies started frequenting the decedent bodies, but the skin was not adversely affected by decomposition (Figure 100a). Three to four-days into decomposition the epidermal skin was peeling and separating from the dermal skin with maggots in the first larval instars occupying the space between the dermal layers (Figure 100b). During four to six-days of active decomposition, fly and other insect activity were abundant, creating noticeable contamination of blood and other biofluids from their movements on the skin. The maggot larvae were also increasing in instar size and overall quantity and had completely occupied the space between epidermal and dermal skin layers (Figure 100c). The visualization of impression details on the decedent skin during this time were slightly obstructed by the contamination of blood and biofluids due to insect activity, primarily the moving maggots beneath the skin that hindered visualization. During six to eight-days of active decomposition, the larval masses were increasing significantly with the maggots mounding and splitting the epidermal skin (Figure 100d) allowing the maggots to emerge in large quantities from the dermal layer. The destruction of the epidermal skin impacted the ability to recover blood impressions, as when the epidermis was significantly damaged the impressions were not able to be recovered. During seven to ten-days of active decomposition most of the epidermal skin was

destroyed leaving behind the dark colored greasy dermal skin (Figure 100e). There were only a few first instar maggots left on the dermal skin, as most of them migrated or matured as decomposition progressed. At this stage in the early decomposition of human decedents, the blood impressions deposited on the skin were no longer able to be recovered.



a) Flies on the Epidermal Skin

b) Fly and Maggots below Epidermal Skin



c) Maggots Separating Epidermal and Dermal Skin

d) Maggot Mass Emerging from Skin

e) Greasy Dermal Skin with Few Maggots

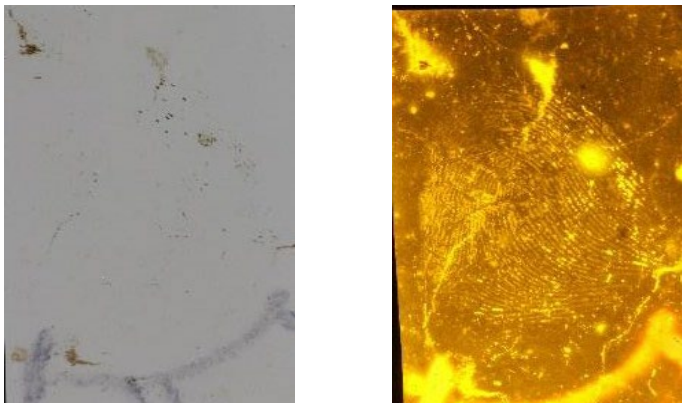
Figure 100: Artifacts of Decomposition on Decedent Skin

If the epidermal skin was intact, the Zar-Pro™ Fluorescent Blood Lifters were able to effectively lift, enhance, and preserve blood impressions from skin during the early stages of decomposition. The Lifters are highly sensitive and inherently fluorogenic in combination with proteinaceous materials when visualized under alternate lighting making them an ideal tool in the

collection of proteinaceous impressions from decedent skin. One of the reasons the Lifters were so effective is that they physically remove the impression from the skin substrate affixing the impression to the Lifters, which help to remove the substrate variables, such as skin color, freckles, moles, and liver spots, and minimize dermal texture that can impede visualization of impression details. Even at the one-day collection interval one of the skin variables, such as the decedent leg hair could be visualized on the Lifter during normal (Figure 101a) and alternate Lighting (101b), but as previously stated the skin variables did not impede the visualization of impression details. At the four-day collection interval, the Lifter picked up a crease on the skin, which is another skin variable which was visualized under normal (Figure 102a) and alternate lighting (102b), which again did not impede visualization of impression details. The most significant factor limiting the effectiveness of the Zar-Pro™ Lifters was not the skin variables but artifacts associated with decomposition. The contamination of the epidermal skin by blood and other biofluids due to insect movements which were also transferred onto the Lifters could impede visualization of the impression details in some cases. However, if the epidermal layer was intact impressions could be recovered, but if only the dermal skin was left impressions were not likely recovered. The greasy residue on the dermal skin contains an abundance of proteinaceous materials, which are transferred onto the Lifters obstructing the visualization of any impression details, even if the impression details were still present on the dermal skin. It should be noted that other proteinaceous residue outside of artifacts of decomposition can also contaminate the Lifters, this was seen in the one-hour collection interval when the sharpie marker used to mark the impression area was visualized on the Lifter under normal (Figure 103a) and alternate lighting (Figure 103b).

a) Normal Lighting (NL) ***b)Alternate Lighting (AL)***

Figure 101: Day 1 Decedent 1- Right Leg Zar-Pro™ Enhanced Impression



a) Normal Lighting (NL) *b) Alternate Lighting (AL)*

Figure 102: Day 4 Decedent 1- Right Leg Zar-Pro™ Enhanced Impression

a) Normal Lighting (NL) *b)Alternate Lighting (AL)*

Figure 103: 1 Hour Decedent 1- Neck Zar-Pro™ Enhanced Impression

Gellifters were used in the trials to lift the dye-stained Amido Black and Hungarian Red impressions from the decedent skin in order to reduce the substrate variables, but the Gellifters were not overly effective. However during the active days of early decomposition, the epidermal and dermal skin was seperating naturally and the Gellifters were damaging the skin thus destroying the impression. In order to preserve the dye-stained impressions, the epidermal skin was removed with a razor blade which allowed for the visualization of the stained impression while minimizing the substrate variables and artifacts of decomposition. The epidermal leg skin of decdent 2 was still intact at the six-day collection interval, thus the epdermal layer was carefully removed from the decdents body (Figures 104a and b). The removal of the hungarian red stained impressions on the dermal skin allowed for improved visualization of impression details (Figure 105a) which was cleaned and secured between two sheeets of glass (Figure 105b) for preservation, protection and ease of visualization. Amido Black dye-stained impressions were also visible on the epidermal skin, in one case the epidermal skin split away from the dermal skin leaving the seperation visible on the blood impression (Figure 106a). As decomposition progressed, the Amido Black dye-stained impressions on the epdermal skin were also carefully removed from the dermal skin but in smaller sections (Figure 106b), as the epidermal skin was less intact than in other body areas. An example of the effects of decomposition can be seen on an Amido Black Stained impression from the one-day collection interval (Figure 107a). As decomposition progressed through the six-day interval a maggot mass can be seen beneath the dye-stained impression (Figure 107b), which impeded the

visualization of the impression details that were previously visible in the stained impression. Thus, the dye-stained impression on the epidermal skin was removed from the dermal skin and cleaned to allow for improved viualization of the impression details (Figure 107c) that were previously obstructed due to the artiifaacts associated with decomposition. The procedure of removing the epidermal skin during the later collection intervals allowed for the improved visulization of the dye-stained impression on skin. Although some of the variables associated with skin, such as hair, were still present, the contours of the body are mitigated along with the artifacts of decomposition, such as the maggot masses thus minimizing the obstruction of impression details and some of the challenges associated with insitu photography conducted in the field. The dye-stained epidermal skin can then be cleaned and preserved to be brought back to the laboratory for photography.

A predicted limitation of the research trials was field photography which proved difficult in the human decedent trials, as photography on skin, even when using a macro lens did not always capture ideal research quality photos. The use of a tripod was also not practical due to the position of the body, curvatures, angles, and contours associated with the impression areas in the various body locations. Some of the other difficulties arose from just being in an outdoor environment with variations of natural lighting and weather conditions, but also lighting conditions associated with the different body areas. Leading to the challenges with capturing impressions on the skin using a macro lens was the presence and movement of insects and larvae as the skin progressed through the early stages of decomposition. This was especially problematic when photographing impressions insitu on the epidermal skin as sub epidermal larval masses were moving beneath the impression area making it difficult to focus the image.

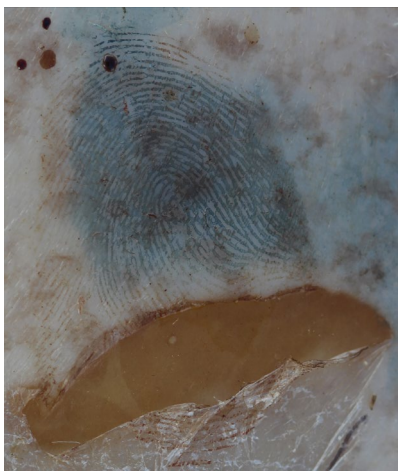
a) Separation of Epidermal and Dermal Skin

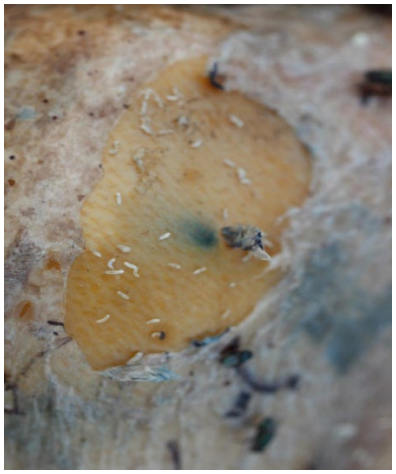
b) Removal of Epidermal Skin- Right Leg

Figure 104: Day 6- Decedent Donor 2

a) Impressions Insitu on Epidermal Skin *b) Epidermal Skin Fixed between Glass*

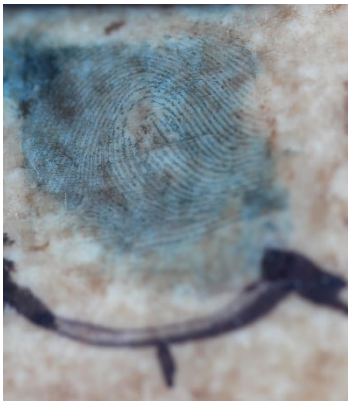
Figure 105: Day 6- Decedent Donor 2 – Hungarian Red Dye-stained Impressions





a) Insitu Skin Split *c) Removal Epidermal Skin*

Figure 106: Day 6 Decedent Donor 2- Amido Black Dye-stained Impressions



a) Insitu 1 Day *b) Insitu 6 Days with Maggot Mass* *c) Removal of Stained Skin*

Figure 107: Day 6 Decedent Donor 1- Amido Black Dye-stained Impressions

Significant Results

The use of an alternate light source, primarily 505nm with an orange barrier filter may be helpful in the detection of latent biofluids, such as semen on decedent skin during the early stages of decomposition. As well as aiding in the detection of visible blood on decedent skin during the later stages of early decomposition, when blood begins to fade and turn latent, however the visualization of biofluids on decedent skin is generally problematic. Lifting impressions from a substrate helps to remove substrate variables which can hinder the visualization of impression details. Human skin has many substrate variables, first off it is a semi-porous substrate which can be problematic for insitu dye staining as the liquid stains will absorb into the skin substrate creating distracting background staining. Other variables of skin are dermal textures, such as creases, scars, hairs, and dermal pattens such as moles, freckles, or liver spots, many of which are darker colored than the skin and can mimic the fine details present in impressions making visualization of impressions insitu on skin more difficult. Zar-Pro™ Fluorescent Lifters were the most effective enhancement method in the trials, due primarily to their fluorogenic properties but also because they lift the proteinaceous

impression from the decedent skin onto the white background of the Lifter for visualization under normal lighting. If the lifted impressions were not visible under normal lighting, the combination of the proteinaceous materials affixed in close proximity to metals embedded in the Lifters excite fluorescent when visualized under alternate lighting. The fluorescent impression can then be visualized on the now darkened background of the Lifter, which again produces an optimal contrast for visualization of impression details. As the Gellifters were not able to effectively lift the dye-stained impression the use of Amido Black and Hungarian Red were limited to insitu enhancement methods. Only insitu enhancement with the use of Gellifters were done in the early collection intervals for the decedent trials as the removal of the epidermis would alter the integrity of the skin for the duration of the research. On the six-day collection interval, the epidermal skin was so fragile that the use of Gellifters were damaging the skin during lifting and as the skin was already being damaged in the lifting process, the dye-stained impressions on the epidermal skin were removed. As the epidermal and dermal skin layers naturally separate during the early stages of decomposition the dye-stained impressions on the epidermal skin can easily be removed from the underlying dermal layer. The removal of the epidermal skin containing the stained impression allows for improved visualization of impression details and helps preserve the impression long term while also improving the quality of insitu photography which can now be conducted in the controlled setting of the laboratory and not just in the field.

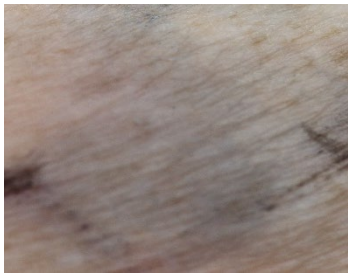
Recovery of Semen Smears from Decedent Donors 1 and 2

Semen smears were deposited onto the pelvic area of the decedent donors. This location was specified in the body diagram as the inner thigh area (LIT) but was changed due to limitations in overall space available on the decedents for the blood impressions. Fifteen semen smears were deposited on to the skin of Decedent Donors 1 (Figures 33c) and 2 (Figure 34c), totaling 30 smears for recovery. The semen smears were enhanced and recovered one-hour after deposition (SC1) at the FARL and on the one-day (S01), three-day (S03) and five-day (S05)collection intervals at FROST. Three smears were enhanced at each collection interval using Amido Black lifted with a white BVDA Gellifters® (EA), Hungarian Red lifted with a black BVDA Gellifters® (EB), and Zar-Pro™ Fluorescent Lifters (EC). Of the 30 deposited impressions only 24 were recoverable due to decomposition of the decedents in the pelvic area, thus six impressions set to be recovered at the seven-day collection interval were not recoverable in these trials. The recovered semen smears were rated by one examiner using only a two-point rating scale of ID (0 = no visible proteinaceous material, no visible ridge detail or 1 = visible proteinaceous material, no visible ridge detail), this was done as impression details were not deposited in the semen smears. As for fluorescent intensity (FI) the previously indicated fluorescent standard was utilized.

After deposition at the one-hour interval semen smears, although latent in nature were visible on the skin substrate due to the glossy composition of the smear itself (Figure 108a and 109a). By the one-day collection interval the glossy sheen of the smear was no longer visible under normal lighting conditions (Figures 110a, 111a, and 112a) through the five-day collection interval. At the one-hour controls, Amido Black dye-stained smears were visible insitu on the decedent skin (108b) and visible on the white background of the Gellifters (Figure108b) under normal lighting. Amido Black stained smears were visible insitu on the decedent skin (Figures 110b, 111b, and 112b) and on the Gellifters (Figures 110c, 111c and 112c) under normal lighting through the five-day collection interval. Hungarian Red stained smears were also visible insitu after staining (Figures 109b, 110d, 111d, and 112d) through five days but none of the stained smears lifted onto the black Gellifters were visible under normal or alternate lighting (Figure 109c) even at the one-hour collection

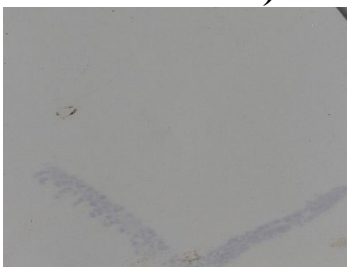
interval. At the one-hour interval, the Zar-Pro™ lifted smears were not visible on the white background of the Lifters (Figure 109d), yet there was visible blood/liquid staining from insect activity visible on the lifters throughout five days (Figures 110e, 111e, and 112e) when visualized under normal lighting. When visualized under alternate lighting the lifted semen smears were readily visible on the Zar-Pro™ Lifters at the one-hour collection interval (Figure 108e and 109e) through five days (110f, 111f, and 112f). Background contamination was present on the lifted semen smears from other biofluids such as from the previously mentioned blood/liquid stains which were noted as early as the one-day collection interval.

a) Insitu Prior Enhancement

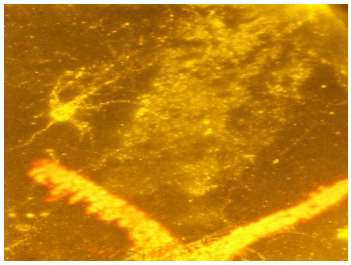


b) Insitu Amido Black

c) Amido Black on Gellifter

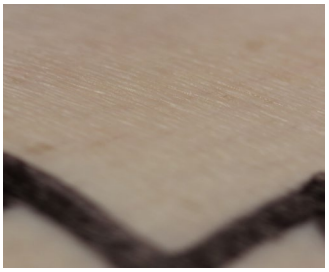


d) Zar-Pro™ NL

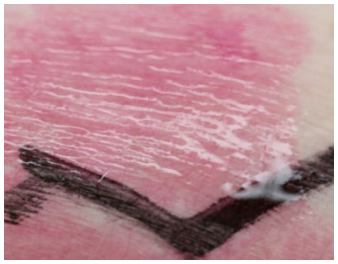


e) Zar-Pro™ AL

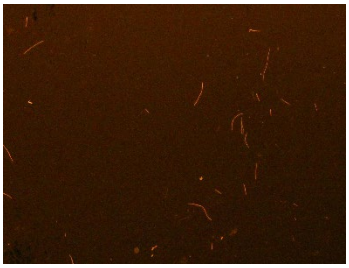
Figure 108 : Decedent Donor 1 Semen Smears- 1 Hour Collection Interval



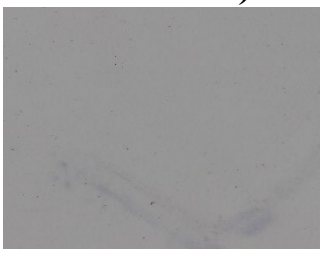
a) Insitu Prior Enhancement



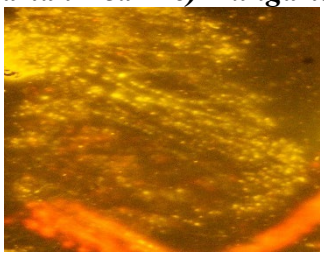
b) Insitu Hungarian Red



c) Hungarian Red on Gellifter



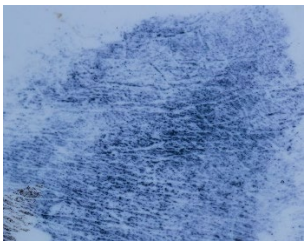
d) Zar-Pro™ NL



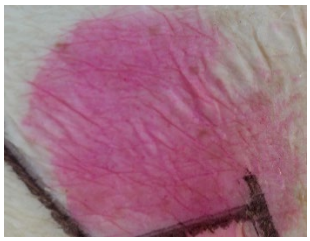
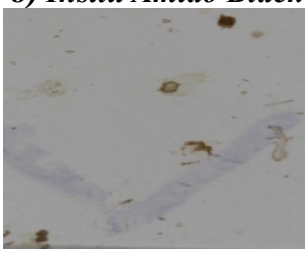
e) Zar-Pro™ AL

Figure 109 : Decedent Donor 2 Semen Smears- 1 Hour Collection Interval





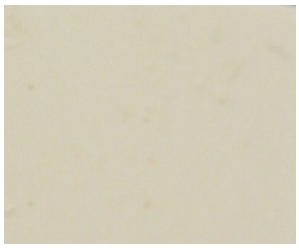
a) Insitu Prior Enhancement *b) Insitu Amido Black* *c) Amido Black on Gellifter*

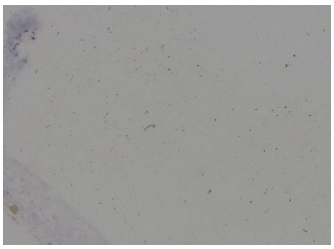
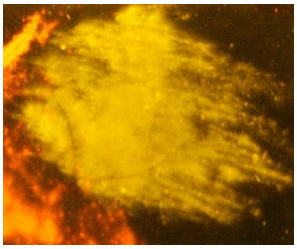

d) Insitu Hungarian Red *e) Zar-Pro™ NL* *f) Zar-Pro™ AL*

Figure 110 : Semen Smears- Day 1 Collection Interval





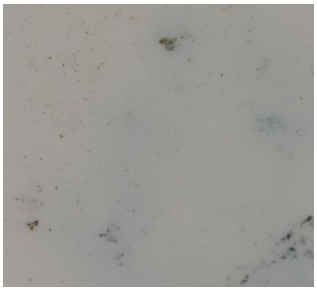
a) Insitu Prior Enhancement *b) Insitu Amido Black* *c) Amido Black on Gellifter*

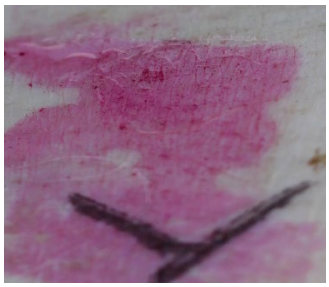
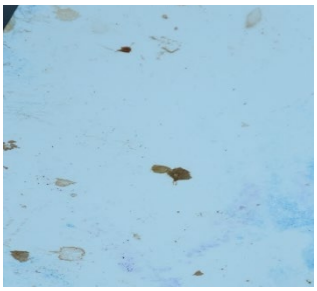
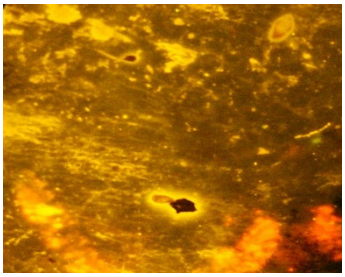
d) Insitu Hungarian Red *e) Zar-Pro™ NL* *f) Zar-Pro™ AL*

Figure 111: Semen Smears- Day 3 Collection Interval





a) Insitu Prior Enhancement *b) Insitu Amido Black* *c) Amido Black on Gellifter*

d) Insitu Hungarian Red *e) Zar-Pro™ NL* *f) Zar-Pro™ AL*

Figure 112: Semen Smears- Day 5 Collection Interval

Significant Results

The semen smears produced similar results to the blood impression trials on decedent skin with all the smears effectively enhanced through the five-day collection interval. The student examiner rated the majority of the enhanced semen smears as a 1 meaning proteinaceous materials were visible after enhancement with the dye stains Amido Black and Hungarian Red and with the Zar-Pro™ Fluorescent Lifters. The fluorescent intensity was also rated under alternate lighting with Zar-Pro Fluorescent Lifters resulting in the highest fluorescence rating from the recovered semen smears. The dye-stains were effective as an insitu enhancement method, but the Gellifters did not effectively lift the stained smears. Even with the fluorogenic properties of Hungarian Red the stained smears on the black Gellifters were not readily visible under alternate lighting. As for the Zar-Pro™ lifted impressions the semen smears were not visible under normal lighting but were readily visible and brightly fluorescent under alternate lighting. It can be concluded that semen will enhance similar to blood thus if a semen impression was deposited on decedent skin it would be enhanced the same as a blood impression through the early stages of decomposition.

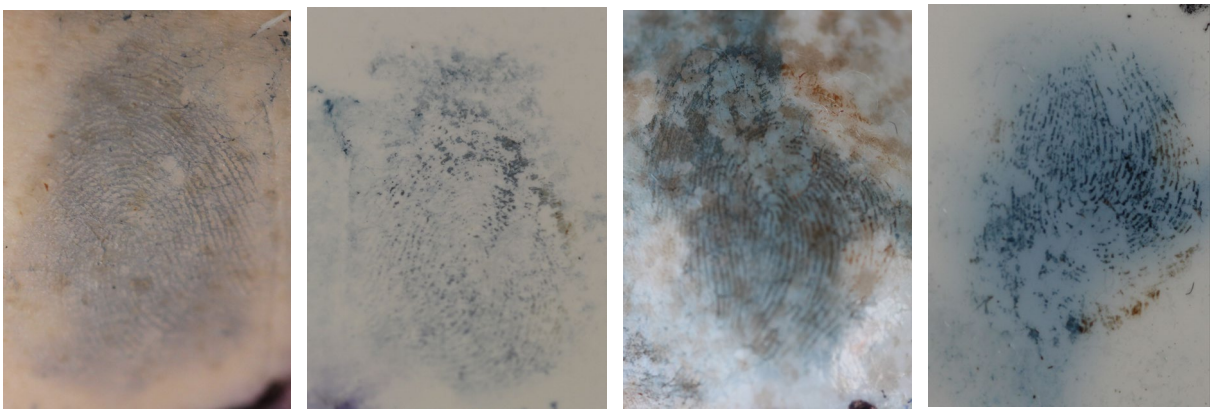
10. Recovery of Blood Impressions from Decedent 3

An assessment of comparability between human Decedent Donors 1, 2, and 3 was conducted which supported the original determination that Donor 3 was not of comparable quality to Donors 1 and 2. Due to antemortem injuries sustained by Donor 3 the decedent's body was contorted and had already starting to decompose, thus there was not adequate surface area on the decedents skin for the deposition of a compete data set. Therefore, the data set for Donor 3 was assessed independently of Donors 1 and 2. Due to the reduction of available skin on D3 and the contorted body positioning, only 33 blood impressions were deposited for the trials which were recovered at the one-hour control (BC1), one-day (B01), two-day (B02), three-day (B03), four (B04) and five-day (B05) collection intervals. The right (LLA) and left (LLA) arms had nine impressions deposited with another 10 on the left leg (LLL) and 5 on the decedents left side (LUC). Non-impression controls were collected from the decedents forehead (LFH) at the one-hour (NC1), one-day (N01), and three-day (NC3) collection intervals using white and black Gellifters (GL) and Zar-Pro™ Lifters (ZP). All three enhancement methods (EA, EB, and EC) were utilized for the recovery of the blood impressions and produced similar results to the blood impression enhanced on decedent donors 1 and 2.

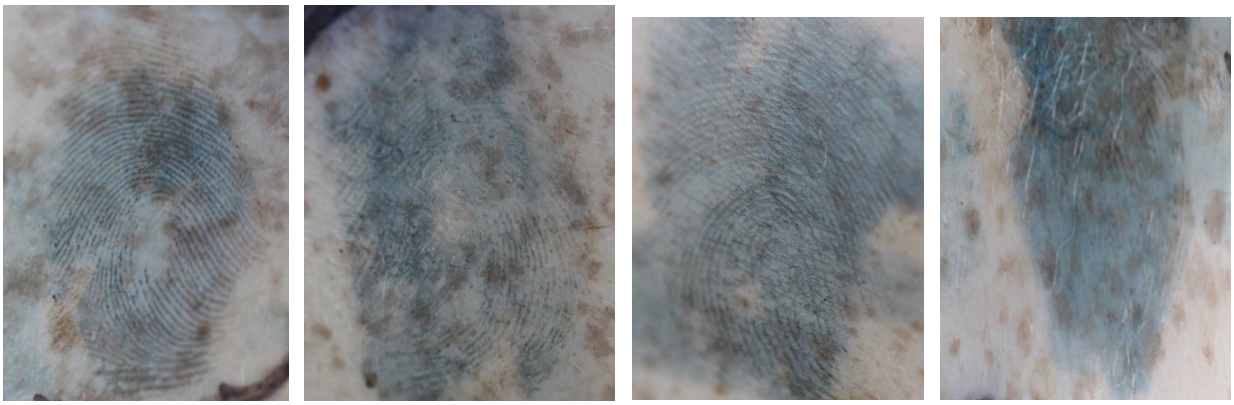
At the one-hour collection interval, there were multiple areas of decomposition noted on decedent donor 3. The abdominal and chest areas, along with both legs were already green from putrefaction and were molding. The rapid onset of decomposition was likely due to the condition of the decedent prior to death, as there were open wounds on the neck from an antemortem tracheotomy, along with medical pads and bandages covering injuries. The decedent also had notable skin slippage over various areas of the body. At this time, Decedent 3 was progressing into

decomposition faster than decedents 1 and 2. Initially decedent 3 was decomposing more rapidly but levelled off and decomposed similarly to the other decedents through the five-day collection interval which was the final day of the impression recovery. After the five-day collection interval, the decomposition of Decedent 3 slowed with decedents 1 and 2 progressing at a more rapid rate, and at the eight-day collection interval Decedent 3 still had intact epidermal skin.

Amido Black was effective as an insitu dye-stain on decedent skin at each collection interval through five days (Figures 113a, c, and e-h) with the details of the blood impression decreasing in quality as the decedent decomposed. The dye-stained impressions were also able to be lifted onto the white Gellifters, but impression details were often lost in transfer. The lifting of the dye-stained impression was most successful at the one-hour (Figure 113b) and one-day (Figure 113d) collection intervals when visualized under normal lighting. Hungarian Red was not as successful as Amido Black as an insitu dye stain on decedent skin as the Hungarian Red stained impressions on the decedent skin often did not show impression details after the first few intervals but allowed for the visualization of proteinaceous materials by staining through the five-day collection intervals (Figures 114a, c, and e-h). The dye-stained impressions were lifted onto the black Gellifters with proteinaceous materials visible under normal lighting (Figure 114b) and some proteinaceous materials visible under alternate lighting (Figure 114d). The Zar-Pro™ Fluorescent Lifters were able to recover blood impression from the decedent skin which were visible under normal lighting through the three-day collection interval (Figures 115a, c, e, g) but only contamination marks from insect activity were visible on the Lifters through the remaining five days (Figures 115i and k). Due to the fluorescent properties of the Lifters, blood impressions were visible when visualized under alternate lighting through the five-day collection interval (Figures 115b, d, f, h, j, and l). However, as decomposition progressed visualization of impression details were obstructed due to background contamination on the Lifters due to artifacts of decomposition (Figure 115l).

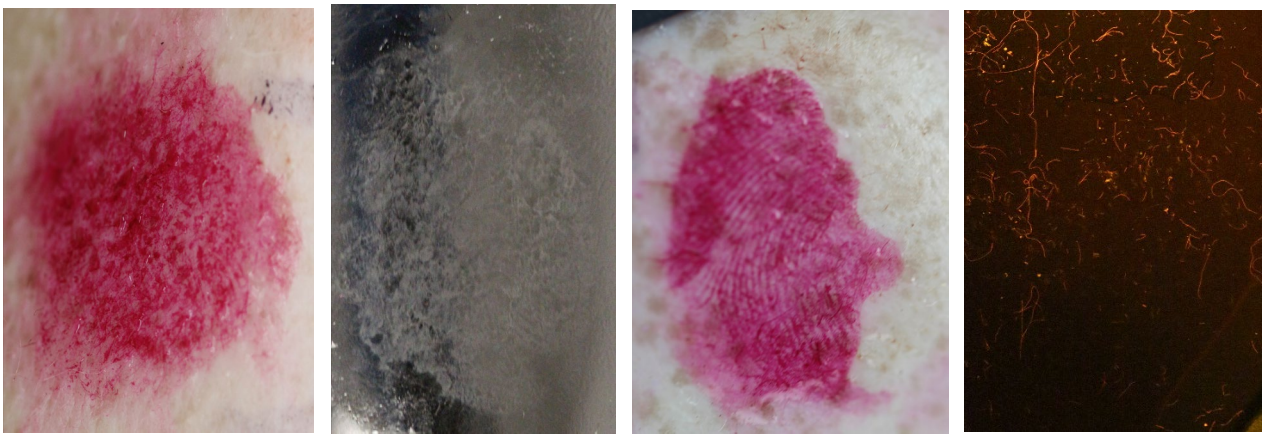


a) One-Hour Insitu ***b) One-Hour Gellifter*** ***c) One-Day Insitu*** ***d) One-Day Gellifter***

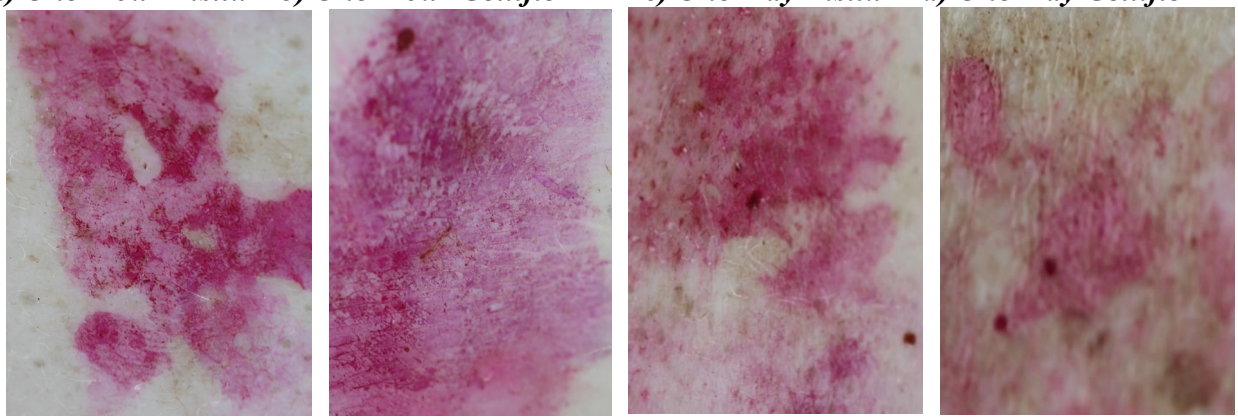


e) Two-Day Insitu *f) Three-Day Insitu* *g)Four-Day Insitu* *h) Five-Day Insitu*

Figure 113: Decedent 3- Amido Black Enhanced Blood Impressions

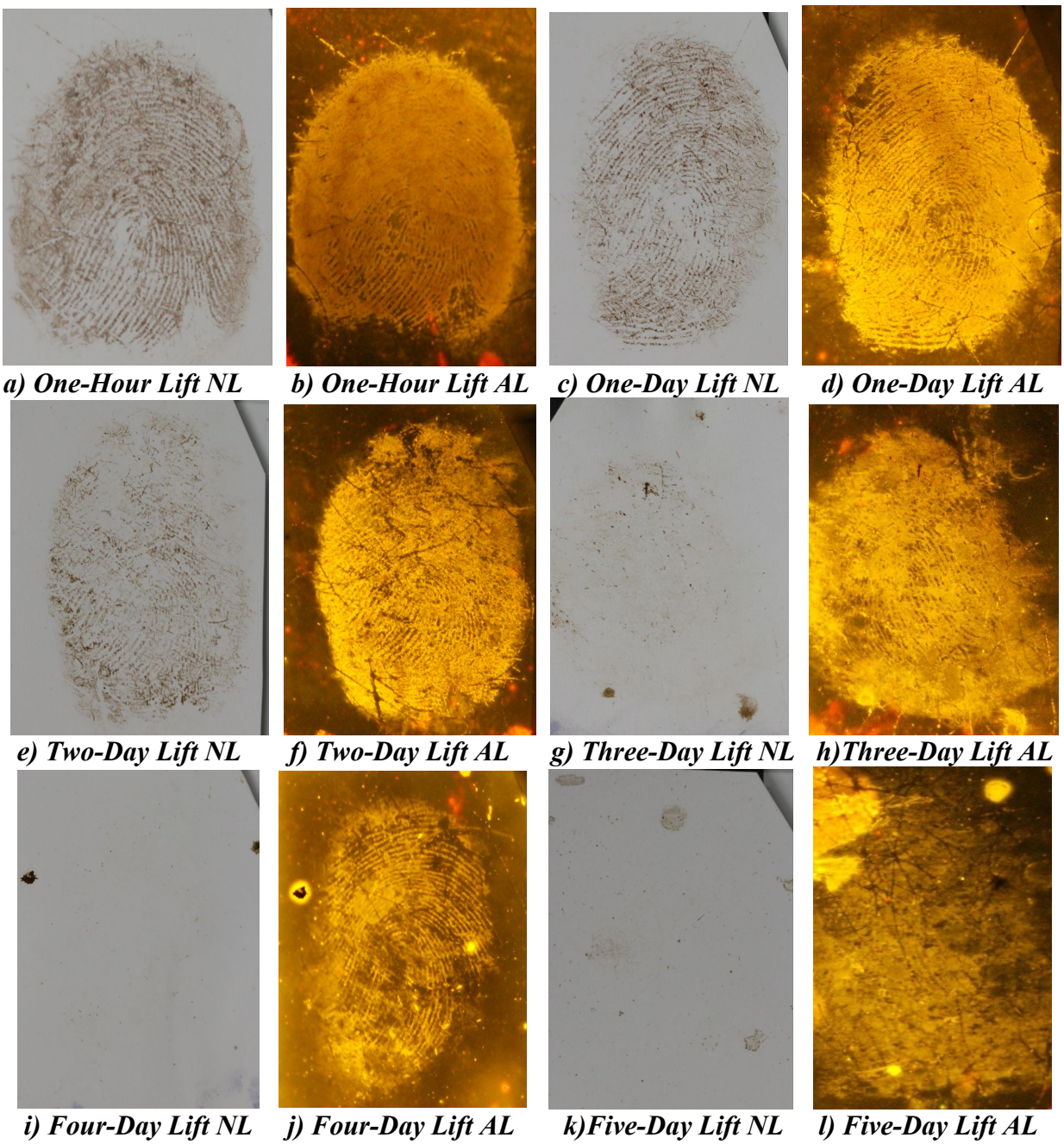


a) One-Hour Insitu *b) One-Hour Gellifter NL* *c) One-Day Insitu* *d) One-Day Gellifter AL*



e) Two-Day Insitu *f) Three-Day Insitu* *g)Four-Day Insitu* *h) Five-Day Insitu*

Figure 114: Decedent 3- Hungarian Red Enhanced Blood Impressions



a) One-Hour Lift NL *b) One-Hour Lift AL* *c) One-Day Lift NL* *d) One-Day Lift AL*

e) Two-Day Lift NL *f) Two-Day Lift AL* *g) Three-Day Lift NL* *h)Three-Day Lift AL*

i) Four-Day Lift NL *j) Four-Day Lift AL* *k)Five-Day Lift NL* *l) Five-Day Lift AL*

Figure 115: Decedent 3- Zar-Pro™ Enhanced Blood Impressions

Significant Results

Decedent Donor 3 allowed for the enhancement of blood impressions using all three recovery methods as outlined in the project but due to the reduced surface area for the deposition of blood impressions, as well as the increased rate of decomposition the donor was not compared to the other decedents. The limited D3 blood impressions were recovered and archived with the project data and produced similar results to the blood impression enhancements from D1 and D2.

11. Environmental and Anthropological Assessment

The data collection portion of the human decedent research was conducted at the Northern Michigan University Center for Forensic Anthropology in Marquette, Michigan. The Center for Forensic Anthropology has three main components: the Body Donation Program, which is what makes the study of human remains at NMU possible, and two teaching/research facilities—the Forensic Research Outdoor Station (FROST), an outdoor taphonomy research site, and the Forensic Anthropology Research Laboratory (FARL), which is the indoor laboratory where skeletal analysis is conducted and skeletal remains of donors' curate.

Total Body Score

In 2005, Megyesi et al. introduced a predictive model for calculating accumulated degree-days (ADD) based on the total body score (TBS) of decomposing human remains. TBS is calculated by breaking the body down into regions (head/neck, torso, limbs) and essentially giving each region a score, based on the observed changes related to decomposition. For example, a fresh body with only early changes visible, would receive a score of 3 for the head/neck; 3 for the torso, and 3 for the limbs, which gives us a TBS (total body score) of 9. In contrast, a body with mostly dry bone, possibly with some desiccated tissue still covering portions of the bone, might receive scores of 10 for the head/neck, torso, and limbs, resulting in a TBS of 30.

Researchers at the NMU CFA have modified Megyesi et al. (2005) published scores to be more appropriate for what is commonly seen at FROST. Decomposition is a nonlinear process, with predictability existing only really in the fact that bodies will progress through early, advanced, and late stages of decomposition. What each of those stages looks like can vary at an individual scale, and certainly varies on a regional scale, which is essentially related to the climate. Observable decomposition changes are not always consistent from climate region to climate region, or even microclimate to microclimate.

The NMU CFA-specific scale was created based on observations collected from a total of 18 donors who were placed out at FROST between 2018 and 2021. For each donor, notes and photographs were taken daily for at least two weeks following placement, or as long as they were in early or active stages of decomposition, which tapered off to once per week, and then once per month, and eventually to once every few months. See Appendix A for the NMU CFA-specific decomposition descriptors for the Head/Neck, Torso, and Limbs, all of which were applied in the assessment of decomposition of the donors used for this project.

On a daily basis, while the Student Researcher from Madonna recovered impressions from the donors according to their established protocol, a team of four NMU students, two undergraduates and two graduate students, simultaneously collected photographs and written notes to document the condition of the donors according to CFA protocol. Each day, a member of the CFA data collection team would return to the FARL facility to upload the images onto the CFA drive where all donor data is stored; the same team member would also upload all written documentation into the CFA Total Body Score database. This information is maintained on a desktop computer that is backed up by duplicating all files onto an external hard drive. The computer is password protected and only users authorized by the CFA director are permitted access to the photo files and database.

Accumulated Degree-Days

Donors 1 and 2 were placed in the outdoor FROST facility on July 20, 2021, and Donor 3 was placed approximately 24 hours later. The delay in his placement was due to time constraints the previous day, and a need to keep Donor 3 refrigerated for as long as possible prior to fingerprint

deposition because of the progression of decomposition. Accumulated degree-days (ADD) have become somewhat of a standard for assessing the progression of the development of biological organisms, or in this case, for assessing the progression of decomposition, in terms of time relative to temperature. Decomposition is highly dependent on temperature and humidity, and the length of time an organism is given to progress through the various stages.

ADDs were calculated for each donor, for each day of data collection, using an R Studio script written by Sage Pletka, an undergraduate student at NMU and a laboratory assistant at the NMU CFA. NMU CFA researchers decided to use 0°C as a base temperature for calculating ADD, following practices established by Megyesi et al. (2005). This is a logical base temperature because it uses freezing as a base, below which bacteria and other biological processes are unlikely to be functional. For the purpose of calculating ADD, NMU CFA researchers used National Weather Service data from the nearest weather station, which is located approximately 20 miles southwest of the FROST site, in Gwinn, Michigan, at the Sawyer International Airport. Although there is a HOBO weather station on-site at the FROST facility, which collects local weather data, the on-site weather station was found to have overwritten the data it collected for several weeks, including the weeks during which data collection for this project occurred.

The ADD for the entire postmortem interval for each donor is presented below. This postmortem interval includes the date of death, ADDs accumulated between death and placement in freezer, ADDs accumulated during the refrigeration period prior to fingerprint deposition and data collection, and the entire data collection period relevant to this research (July 20, 2021 through July 30, 2021). Photographic documentation of the progression of decomposition for each donor can be reviewed in the project appendices (Appendix G).

Donor 1 (D1)

Days between death and placement in freezer: less than 1 (same day)
Average storage temperature between death and placement in freezer: 2°C
Date moved to refrigerator: 7/9/2021
Date fingerprints placed: 7/20/2021
Date of FROST placement: 7/20/2021

Accumulated Degree-Days: Donor 1 ND = No Data (in storage)				ADD	Score			
					HN	T	L	TBS
ADDs between Death and Refrigerator Placement:				2	1	1	1	3
Date	High Temp °C	Low Temp °C	Avg Temp °C		ND	ND	ND	ND
7/9/21	4	4	4	6	1	1	1	3
7/10/21	4	4	4	10	ND	ND	ND	ND
7/11/21	4	4	4	14	ND	ND	ND	ND
7/12/21	4	4	4	18	ND	ND	ND	ND
7/13/21	4	4	4	22	ND	ND	ND	ND
7/14/21	4	4	4	26	ND	ND	ND	ND

7/15/21	4	4	4	30	ND	ND	ND	ND
7/16/21	4	4	4	34	ND	ND	ND	ND
7/17/21	4	4	4	38	ND	ND	ND	ND
7/18/21	4	4	4	42	2	2	2	6
7/19/21	4	4	4	46	ND	ND	ND	ND
7/20/21	21.1111	8.3333	15.0556	61.0556	2	2	2	6
7/21/21	22.2222	5.5556	12.7778	73.8334	2	3	2	7
7/22/21	23.8889	13.8889	17.3889	91.2223	4	4	3	11
7/23/21	26.1111	15.5556	19.5556	110.7779	5	5	3	13
7/24/21	28.8889	17.7778	21.8889	132.6668	5	5	3.5	13.5
7/25/21	30.0000	14.4444	20.1667	152.8335	6	6	4	16
7/26/21	30.0000	14.4444	19.6111	172.4446	6	7	5	18
7/27/21	25.0000	14.4444	17.8889	190.3335	7	8	6	21
7/28/21	27.7778	11.1111	17.5000	207.8335	7	8	7	22
7/29/21	20.0000	11.6667	17.5556	225.3891	8	8	8	24
7/30/21	20.0000	6.1111	12.5556	237.9447	8	8	8	24

Donor 2 (D2)
Days between death and placement in freezer: 1
Average storage temperature between death and placement in freezer: 4ºC
Date moved to refrigerator: 7/9/2021
Date fingerprints placed: 7/20/2021
Date of FROST placement: 7/20/2021

Accumulated Degree-Days: Donor 2 ND = No Data (in storage)				ADD	Score			
					HN	T	L	TBS
ADDs between Death and Refrigerator Placement:				4	2	2	2	6
Date	High Temp ºC	Low Temp ºC	Avg Temp ºC		ND	ND	ND	ND
7/9/21	4	4	4	8	2	2	2	6
7/10/21	4	4	4	12	ND	ND	ND	ND
7/11/21	4	4	4	16	ND	ND	ND	ND
7/12/21	4	4	4	20	ND	ND	ND	ND
7/13/21	4	4	4	24	ND	ND	ND	ND
7/14/21	4	4	4	28	ND	ND	ND	ND
7/15/21	4	4	4	32	ND	ND	ND	ND
7/16/21	4	4	4	36	ND	ND	ND	ND
7/17/21	4	4	4	40	ND	ND	ND	ND
7/18/21	4	4	4	44	2	2	2	6
7/19/21	4	4	4	48	ND	ND	ND	ND

7/20/21	21.1111	8.3333	15.0556	63.0556	2	3	4	9
7/21/21	22.2222	5.5556	12.7778	75.8334	2	3	4	9
7/22/21	23.8889	13.8889	17.3889	93.2223	3	4	5	12
7/23/21	26.1111	15.5556	19.5556	112.7779	4	5	5	14
7/24/21	28.8889	17.7778	21.8889	134.6668	5	6	5.5	16.5
7/25/21	30.0000	14.4444	20.1667	154.8335	5	7	5.5	17.5
7/26/21	30.0000	14.4444	19.6111	174.4446	6	7	6.5	19.5
7/27/21	25.0000	14.4444	17.8889	192.3335	7	7	7	21
7/28/21	27.7778	11.1111	17.5000	209.8335	7	8	8	23
7/29/21	20.0000	11.6667	17.5556	227.3891	8	8	8	24
7/30/21	20.0000	6.1111	12.5556	239.9447	8	8	8.5	24.5

Donor 3 (D3)

Days between death and placement in freezer: 3
Average storage temperature between death and placement in freezer: 4ºC
Date moved to refrigerator: 7/9/2021
Date fingerprints placed: 7/21/2021
Date of FROST placement: 7/21/2021

					Score			
Accumulated Degree-Days: Donor 3 ND = No Data				ADD	HN	T	L	TBS
ADDs between Death and Refrigerator Placement:				12	2	2	2	6
Date	High Temp ºC	Low Temp ºC	Avg Temp ᶜ		ND	ND	ND	ND
7/9/21	4	4	4	16	2	2	2	6
7/10/21	4	4	4	8	ND	ND	ND	ND
7/11/21	4	4	4	8	ND	ND	ND	ND
7/12/21	4	4	4	8	ND	ND	ND	ND
7/13/21	4	4	4	8	ND	ND	ND	ND
7/14/21	4	4	4	8	ND	ND	ND	ND
7/15/21	4	4	4	20	ND	ND	ND	ND
7/16/21	4	4	4	36	ND	ND	ND	ND
7/17/21	4	4	4	44	ND	ND	ND	ND
7/18/21	4	0	2	50	2	3	3	8
7/19/21	0	0	0	52	ND	ND	ND	ND
7/20/21	0	0	0	52	ND	ND	ND	ND
7/21/21	22.2222	5.5556	12.7778	64.7777778	2	3	4	9
7/22/21	23.8889	13.8889	17.3889	94.9444445	3	3	4	10
7/23/21	26.1111	15.5556	19.5556	131.888889	4	3	5	12
7/24/21	28.8889	17.7778	21.8889	173.333333	5	4	5	14
7/25/21	30.0000	14.4444	20.1667	215.388889	5	5	5.5	15.5
7/26/21	30.0000	14.4444	19.6111	255.166667	6	6	5.5	17.5
7/27/21	25.0000	14.4444	17.8889	292.666667	7	7	6.5	20.5

7/28/21	27.7778	11.1111	17.5000	328.055556	7	7	7	21
7/29/21	20.0000	11.6667	17.5556	363.111111	8	8	8	24
7/30/21	20.0000	6.1111	12.5556	393.222222	8	8	8	24

Information on environmental and anthropological variables from this study was assessed to provide a baseline relationship between the effectiveness of recovering impressions from decedent skin during the early stages of decomposition. Anthropological observations were recorded throughout the trials to include Donor Body Mass Index (BMI), daily body temperature and TBS. Environmental observations were also recorded to include ambient temperature, humidity, and rainfall, as well as any observations regarding condensation on decedent skin. Additionally, insect activity was also noted to provide information regarding the stages of decomposition as the body progressed from fresh to active decomposition.

The BMI for D1 was 28.0, D2 was 28.2 and D3 was 18.7, as Donors 1 and 2 had similar BMI they were deemed comparable for purposes of the trials (Figure 116). It was evident from the beginning of the study that D3 would not be comparable to the other donors, specifically as the BMI of the donors may also influence the decedents TBS. Daily body temperatures were collected for the decedents at the one-hour controls (BC/SC1/NC1) with D1 and 2 at 4 ºC and D3 at 0ºC. The decedents daily body temperatures rose to 21.9 ºC at the four-day (B04/S04) collection interval ending at 12.6 ºC at the nine-day (B09/S09) collection interval. Overall photographs of the decedents were taken daily both before and after the collection intervals (Figure 116) to allow for additional assessments to be made throughout the ten days of trials. It is important to note that the decedent donors were not completely fresh at the time of deposition of the blood impressions and semen smears and had already begun active decomposition. At the one-day (B01/S01/N01) collection interval D1 had pink lividity but did not have any other noticeable color change to the skin, D2 had a darker pink coloration to the skin with noticeable bloating, and D3 had a slight pink skin color and had several areas of mold due to the skin rotting, which was mostly around the medical equipment still in place on the decedent. The decedents continued to progress with skin colorations due to decomposition continuing through the three-day (B03/S03/N03) collection interval. At the four-day collection interval (B04/S04) the D1 and 2 were both bloated, and the skin was beginning to dry out with a clear separation occurring between the epidermal and dermal skin layers. During the five-day (B05/S05/N05) collection interval all three decedents had mold visible on the skin in various areas and the skin was beginning to de-flesh as the bodies were advancing through decomposition. By the seven-day (B07) collection interval D1 had a jump in TBS which correlated to the overall look of the body which began to show signs of mummification. The epidermal skin was no longer present in most body areas with a distinct brown color present with a greasy coating on the dermal skin layer. D2 also progressed into this condition by the eight-day (B08) collection interval and by the nine-day (B09) collection interval both decedents were covered with the dark brown greasy dermal skin layers as the epidermis was no longer present (Figure 116). D3 did not achieve this level of decomposition until after the ten-day (B10) collection interval. Upon the completion of the trials the decedents were placed under an isolated tent to prevent mummification.

	Donor 1	Donor 2	Donor 3

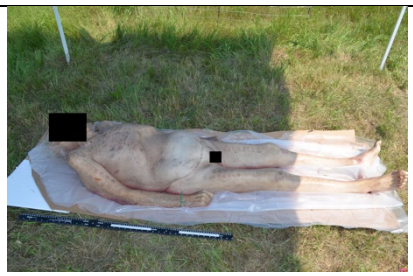
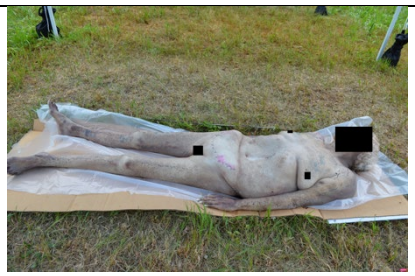
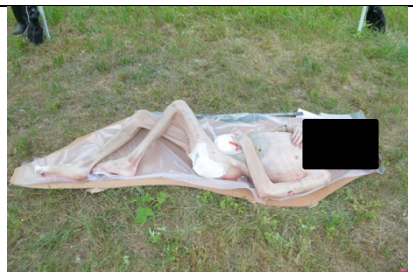
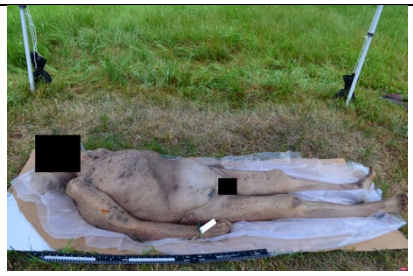
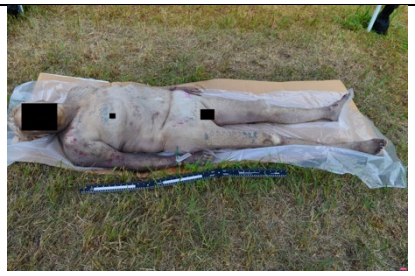
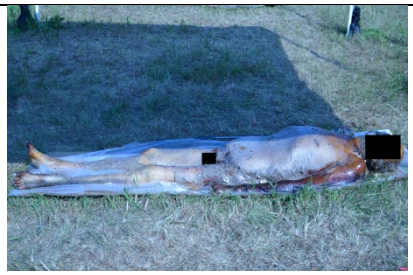
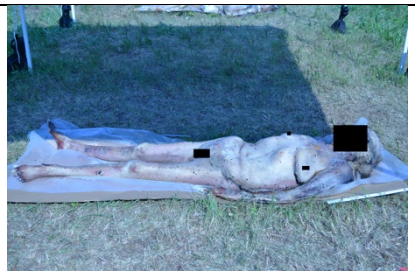
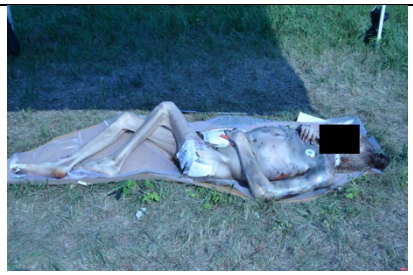
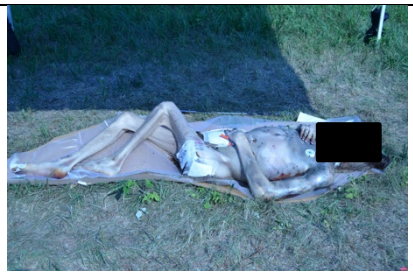
Day 1			
Day 3			
Day 5			
Day 7			

Figure 116: Comparison Amongst Decedent Donors

Collection Interval Summer 2021	Date	High Temp (C)	Low Temp (C)	Average Temp (C)	Humidity (%)	Precipitation (in)	Barometric Pressure (in)
BC1/SC1	7/20	24.5	12.3	18.6	74	0	30.13
B01/S01	7/21	22.2	10.8	16.4	61	0	30.20
B02/S02	7/22	26	16.8	21.4	52	0	30.15
B03/S03	7/23	28.7	17.0	23.1	74	0.37	30.02
B04/S04	7/24	28.7	21.5	25.3	72	0.21	29.76
B05/S05	7/25	30.7	19.0	24.7	45	0	29.85
B06/S06	7/26	26.4	18.5	22.5	51	0	29.93
B07/S07	7/27	24.6	16.2	20.3	75	0.12	30.01
B08/S08	7/28	29.2	14.7	21.9	71	0.08	29.99
B09/S09	7/29	21.8	15.8	18.6	78	0	29.98

Table 5: Weather Data from Human Decedent Trials

The weather conditions were averaged and recorded for each daily collection interval with temperatures ranging from a low of 10.8 °C to a high of 30.7°C over the course of the human decedent trials in Marquette, Michigan. During the trials, the weather in Marquette, Michigan was cooler at night but warmer in the day due to the summer season and had humidity ranging from 45-78% with the barometric pressure averaging 30 inches (Table 5). The most important weather variable associated with the ability to recover blood impressions and semen smears from the decedent skin was the amount of precipitation. The three-day collection interval (B03/S03/N03) had the highest volume of precipitation at 0.37inches of rainfall, followed by 0.21inches at the four-day (B04/S04) collection interval (Table 5). At both the three-and four-day collection intervals the amount of rainfall was noted due to the detrimental effect on impression recovery (Figures 118 and 119). The seven-day (B07/S07/N07) had only 0.12 inches of rainfall and the eight-day (B08/S08) collection interval had 0.08 inches (Table 5), which did not have as great effect on the impression recovery, possibly due to the more advanced stages of decomposition as impression recovery was being affected at this time by the deteriorating skin condition.

Significant Results

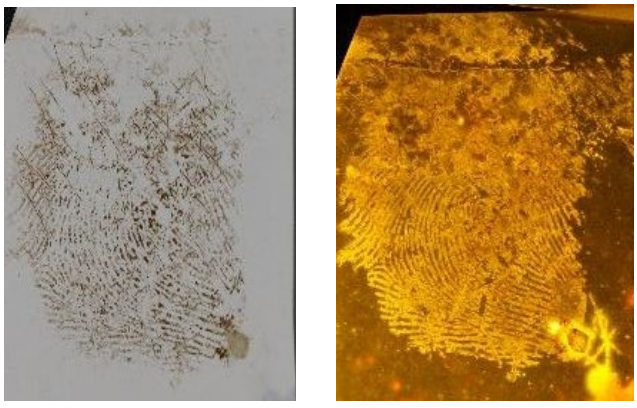
In assessing the variation in the decedent donors, the original decision to separate D3 from the trials was important as throughout the ten days of trials D3 was not comparable to D1 and D2. This was evident from the beginning of the trial due to BMI and lack of overall surface area for the deposition of impressions and continued throughout the collection intervals with the daily assessment of the TBS and Body temperature. Impression recovery from the skin of human decedents during the early stages of decomposition will be affected by anthropological and environmental variables as decedents progress through the early stages of decomposition. Other than precipitation these variables, however, do not by themselves prevent the recovery of blood impressions or semen smears from decedent skin even over the course of several days. Additional research may need to be conducted to improve the assessment and application of the knowledge obtained in this study to casework, especially as environmental conditions will vary throughout geographical areas.

V. Environmental Effect of Precipitation on Impression Recovery

Precipitation causing moisture on the skin of human decedents or fetal pigs did not necessarily destroy blood impressions unless the rainfall or snowfall came down with enough force to wash away or destroy the impressions. Thus, blood impression with moisture on the skin could still be visible but were often destroyed by smearing or smudging during the lifting process. This was seen in human decedent trials as a result of condensation on the decedent skin due to weather conditions, such as morning condensation covering the decedent skin. This was also observed during the deposition of the blood impressions onto the decedent skin at the start of the trials. This was more pronounced on decedent donor 2 due to the warming of the skin after removal from the refrigerators, even though the depositions were conducted in a refrigerated laboratory the bodies were still sweating which was creating moisture on the skin. At the one-hour collection interval, the Zar-Pro™ lifted impressions appeared un-affected by the body sweating under normal lighting (Figure 117a), but the dispersion of blood was visible under alternate lighting (Figure 117b). Due to weather conditions during the early stages of decomposition, blood impressions were affected by precipitation on the skin at the three-day and four-day collection intervals. At the three-day interval, water drops can be seen on the Lifter under normal lighting conditions (Figure 118a), when visualized under alternate lighting conditions the water drops were still visible, along with blood diffusion around the impression area (Figure 118b). At the four-day collection interval, there were no ridge details visible under normal lighting (Figure 119a) but a diffused impression with ridge details were visible under alternate lighting (Figure 119b).

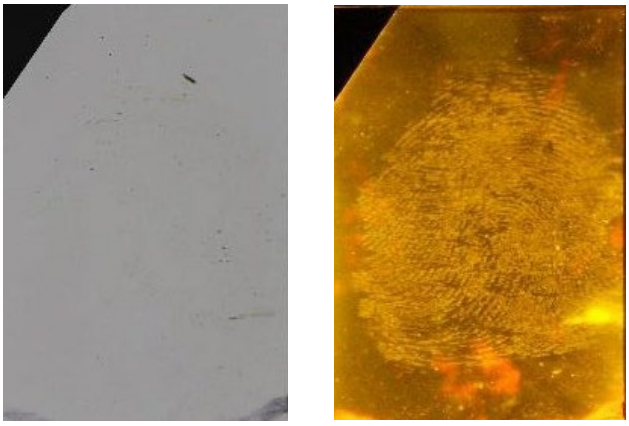
a) Wet Impression NL ***b) Wet Impression AL***

Figure 117: One Hour Decedent 2- Left Arm Zar-Pro™ Enhanced Impression



a) Wet Impression NL *b) Wet Impression AL*

Figure 118: Day 3 Decedent 2- Left Arm Zar-Pro™ Enhanced Impression



a) Wet Impression NL *b) Wet Impression AL*

Figure 119: Day 4 Decedent 2- Left Arm Zar-Pro™ Enhanced Impression

As a result of the noted detrimental effect of precipitation on the recovery of blood impressions from skin, a series of trials were conducted at the MUFSRF lab to minimize the destruction caused by rain or snowfall in future studies. Preventing the destruction of blood impressions from wet skin during recovery with Zar-Pro™ Fluorescent Lifters by drying the impression areas to remove moisture prior to lifting to prevent this issue was the focus of the project.

1. Deposition Parameters

The skin used for these trials was the left and right forearms of a student researcher. The forearms were cleaned with water and then dried with paper towel prior to impression deposition to prevent alterations from environmental contaminants. The deposition parameter set for the fetal pig and human decedent trials were also followed for the deposition of blood in these trials. A series of ten blood impressions (five on each arm) were deposited onto the forearm and then left to dry for 24 hours before use in the trials.

2. Misting Parameters and Surface Area Calculations

A small spray bottle (SEOH) was selected to apply moisture to the impression area as it produced a consistent and fixed stream when sprayed using a pump applicator. The surface area of the mist output from a single spray was calculated by adding blue food color to water and spraying the bottle onto a paper shop towel placed .5 inches from the nozzle. The parameter, length (in) x width (in) of the spray mist (Figure 120) was then measured and then averaged over ten trials, determining the surface area covered by the spray mist was 4.78 in^2. The distance of the spray nozzle to the substrate was also assessed to allow for variation of spray output and its effect on blood impressions deposited on human skin. The initial distance of 1.5 inches was deemed too close as the water was very concentrated and intense at this distance, thus highly destructive to the impressions. Therefore, the spray nozzle distance from the substrate was positioned to 6 inches to allow for a dispersal of the water on the impression without disrupting the impression details. A single spray of water at 6 inches away was calculated to have a surface area of 33.69 in^2.

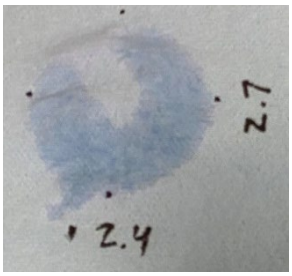


Figure 120: Measuring Spray Area

The output volume of each spray and the designated levels of mist intensity were established for the trials. Spray volumes per mist were quantified by collecting the water sprayed from the bottle into a petri dish from 6 inches away. Pooled water in the petri dish was then removed with a P200 pipette and the volume was recorded. Mist intensity was designated into three levels: light, medium, and heavy intensity. To quantify intensity of water output a series of ten trials were conducted and the output water volume and resultant intensity were recorded. Light intensity misting was defined as one pump sprays with a resultant water volume of 146.1µL, medium intensity was two sprays with an average volume of 306.3µL, and high intensity had three sprays with an average volume of 402.3µL. Water volumes over 500µL applied this close to blood impressions on skin were found to be destructive to the impression.

3. Drying Parameters

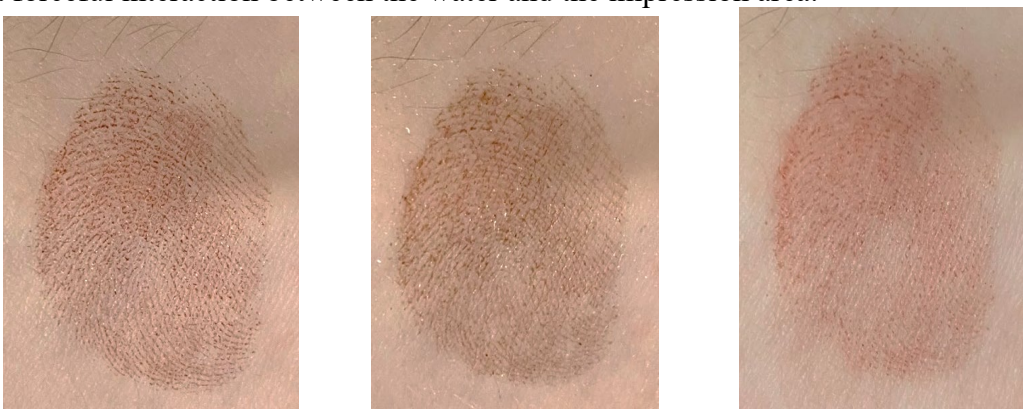
A blow dryer (Revlon RV417, 1600-watt, compact travel dryer) was selected to dry the water-misted blood impressions before lifting in the trials. The dryer had two heat/speed settings, low (125 volts) and high heat (250 volts). The low heat setting was selected to keep the intensity of heat to a minimum, to not alter the integrity of the blood impression. The amount of heat emitted from the low setting was quantified using a laser thermometer (General Tools IRT3) utilizing three methods at a distance of 6 inches. First, the blower heat output was measured at 0 and 30 seconds of use. Secondly, air temperature was measured by blowing air into a small cup for 30 seconds and measuring the change in temperature. Thirdly, the blow dryer heat output was measured by blowing heat onto a lab bench top for 30 seconds and recording the change in temperature. All recorded temperatures were then averaged to determine the heat produced on the low setting at 125 volts for 30 seconds which was -11.6°C. Overall dry times were determined based on spray volume, one spray required a 10 second dry time, whereas 2 sprays required 15 seconds and three sprays needed 20 seconds to dry excess moisture.

4. Collection Intervals and Impression Assessment

A series of trials were conducted to assess the effectiveness of heat drying the moisture around blood impression before using Zar-Pro™ Fluorescent Lifters to improve impression recovery. The collection intervals were determined based on spray intensities: Low, Medium, and High. Blood impressions deposited on the forearm were dried for varying intervals up to 24 hours. A series of blood impressions were left on the arm for various times, and it was determined that impressions deposited for less than 24 hours were not as stable when misted as impressions that dried on the skin for over 24 hours. Thus, in the remainder of the trials blood impressions were left on the skin for over 24 hours before misting. The first 24-hour trial included depositing ten impressions, five on each forearm. The Zar-Pro™ lifted impressions were then assessed based on impression details and fluorescent intensity using the provided scale standards to determine efficacy.

5. Misting Trial Results

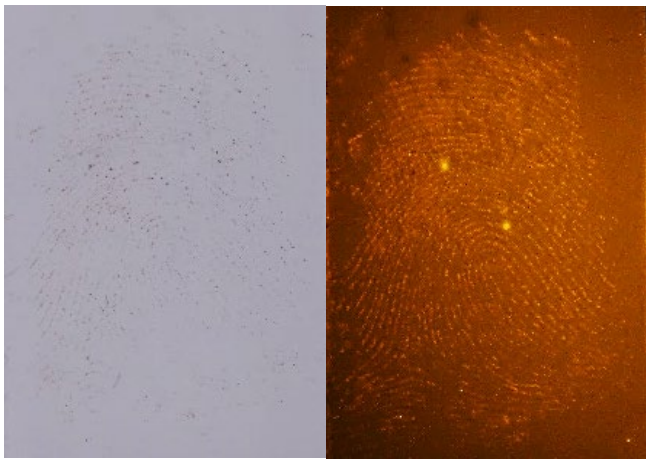
Blood impressions prior to misting showed visible ridge detail (Figure 121a) after misting impression details were still present and moisture around the area was noticeable (Figure 121b) while after the misting impression details began to deteriorate, slightly washing away details as the impression dried (Figure 121c). The destruction of the impression due merely to the drying of an impression that had been wet appeared to be more prevalent with blood impressions that sat insitu on the skin for less than 12 hours. Impressions left insitu on the skin for over 24 hours appeared to be more stable and long lasting. Thus, it was noted that impressions on the skin that were left for a day before being exposed to moisture from misting were not as affected by precipitation, especially if it wasn't a forceful interaction between the water and the impression area.



a) Insitu 30 mins-Pre Misting ***b)After Misting*** ***c)After Drying***

Figure 121: Misting Blood Impressions

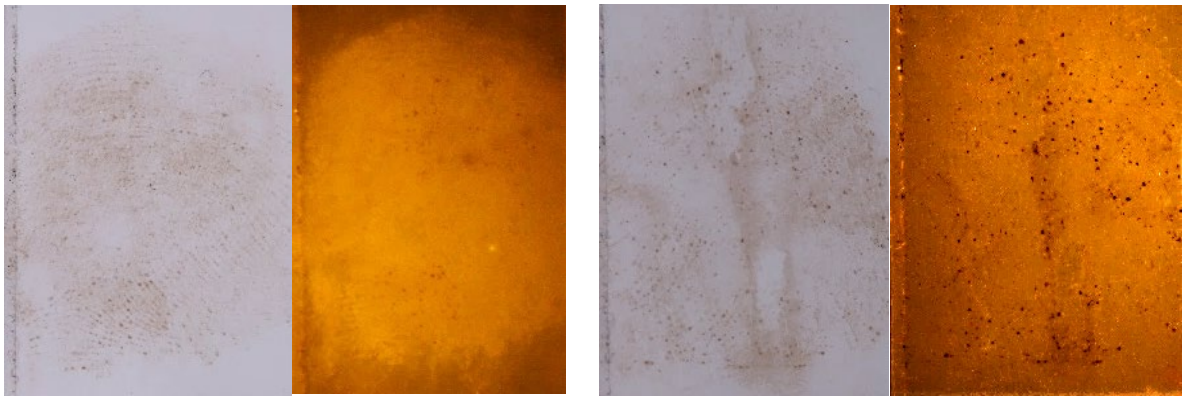
Impressions left insitu on skin for twenty-four hours, were then exposed to misting under six different conditions before being lifted with the Zar-Pro™ Fluorescent Lifters. Five impressions on the right forearm were lifted without any misting or drying to act as controls for the study (Figure 122a and b). The first impression was misted with one spray, dried for ten seconds with a blow dryer, and then lifted. The second was misted with two sprays, dried for fifteen seconds with a blow dryer, and then lifted. The third was misted with three sprays, dried for 20 seconds with a blow dryer, and then lifted. The fourth was misted with two sprays and lifted wet. And the fifth was misted with three sprays and lifted wet. To further test the parameters a second set of trials were conducted to compare wet and dry lifts. Ten blood impressions were again deposited onto the left and right arms and left for 24 hours before the five impressions on the right forearm, were sprayed three times with the mister and then lifted immediately with Zar-Pro™ Lifters while still wet. The five impressions on the left arm were each sprayed three times then dried with a blow dryer for 20 seconds before being lifted with Zar-Pro™ Lifters.



a) Normal Lighting b) Alternate Lighting

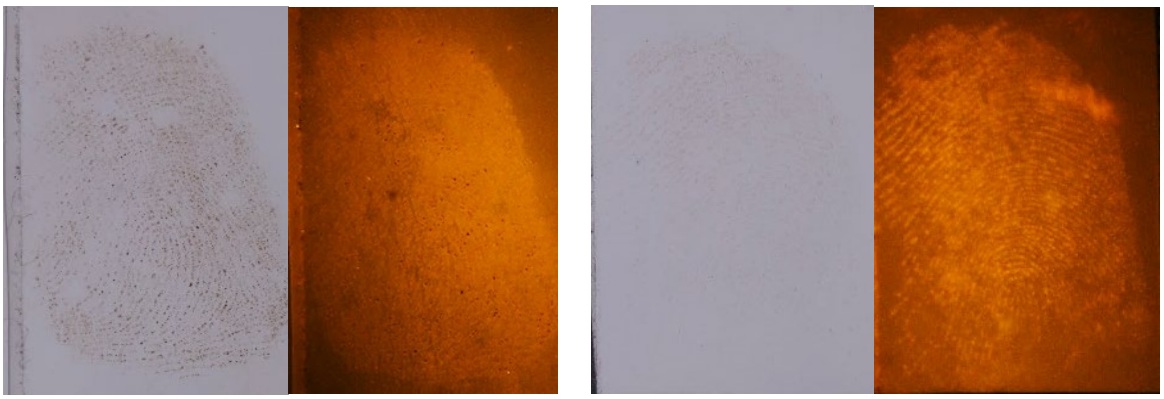
Figure 122: Zar-Pro™ Control

The process of lifting wet blood impressions with the Zar-Pro™ Fluorescent Lifters resulted in the destruction of impression details due to smearing and smudging of the blood during the application of the Lifter. Blood impressions that were sprayed three times (heavy mist) and lifted wet were smeared and smudged causing destruction to the impression which can be seen both under normal (Figure 123a and c) and alternate lighting (Figure 123b and d). Drying the moisture surrounding the impression area with a blow dryer to rid the area of moisture has shown to be an effective method to preserve impression detail through the lifting process. Impressions that were sprayed three times (heavy mist) and lifted after drying (20 Seconds) with a blow dryer showed visible impression details under normal lighting (Figure 124a and c), although some of the impression details were faint. The lifted impressions were also visualized under alternate lighting (Figure 124b and d) with impression details visible, however fluorescent impression details do appear to be blurred in some areas. This is due to diffusion of the proteinaceous materials in the blood while the impression was wet which can be seen in the fluorescent impression but not under normal lighting conditions.



a)Normal Lighting b) Alternate Lighting c)Normal Lighting d) Alternate Lighting

Figure 123: Wet Impressions Lifted with Zar-Pro™



a)Normal Lighting b) Alternate Lighting c)Normal Lighting d) Alternate Lighting

Figure 124: Wet Impression Dried then Lifted with Zar-Pro™

Significant Results

Through these trials it was determined that blood impressions are not stable on the skin substrate until they remain insitu from anywhere between twelve to twenty-four hours. It appears that the longer the blood remains insitu on the skin increases the stability of the impressions, thus impressions left insitu are not as easily destroyed from precipitation. The force at which the precipitation contacts the impression area, as well as the distance from the impression to the water source will affect the condition of the impressions. Even a heavy mist if not contacting the impression areas with a strong force will not have a detrimental effect on the integrity of the blood impressions, thus wet impressions can still have viable impression details even under pooled water. A simple method of drying a wet impression with a hairdryer prior to lifting was tested, and the resulting lifted impressions were analyzed to evaluate the efficacy of the drying method. As a result of these trials, it was concluded blood impressions affected by environmental precipitation should be dried to eradicate moisture from the impression area prior to lifting with Zar-Pro™ Fluorescent Lifters. As these trials indicate drying can be done with the use of a blow dryer to improve the effectiveness of the Lifters, as well as the overall quality of the lifted impression, thus an improved analysis could be performed.

VI. Future Focused Workforce Initiative

As part of the future-focused workforce initiative, a total of twenty student researchers studying forensic science at Madonna University have been trained in various aspects of the project from the optimization of deposition parameters, the detection and documentation of impressions in situ, as well as the physical and chemical enhancement and the proper collection and packaging of impression-based evidence. Many of these students were also involved in the environmental assessment and resultant data analysis, including serving as student examiners for the Cohens Kappa statistical assessment. Ten student researchers worked on the Fall 2020 and Fall 2021 fetal pig trials on the campus of Madonna University. Additionally, five of the student researchers travelled to Northern Michigan University to participate in the summer 2021 human decedent trial, along with an additional four student researchers studying at Northern Michigan University.

Dissemination of Research Findings

As a result of this research, two student researchers presented preliminary data from the Human Decedent Trials at the International Association of Identification (IAI) annual conference, August 2021, Nashville, Tennessee. The poster presented by Kristen Szabelski and Sarah Holton titled,

Methods to Enhance and Preserve Blood Impressions from the Skin of Decedents During the Early Stages of Decomposition was awarded first place in the IAI Student Poster Contest. An oral presentation was given by the project PI and Co-PI titled, "Recovering blood impressions and semen smears for human decedents during the early stages of decomposition" and a poster was presented by student researchers at the International Association of Identification annual conference, August 2022, Omaha, Nebraska. Two students presented a poster at the Forensic Technology Center of Excellence (FTCoE) in a virtual presentation during forensic science week, September 2022 and another two students presented a poster at the 6th NIJ Forensic Research and Development Symposium at the PITTCON Conference, March 2023, Philadelphia, Pennsylvania. Three students will be presenting at the Michigan Academy of Science Arts and Letters annual conference, Lawrence Technological University, Southfield, Michigan in March 2024 and at the Madonna University Symposium of Research, Scholarship, and Creativity, April 2024, Livonia, Michigan. Abstract applications were submitted for an oral presentation at the 2023 and 2024 NIJ Forensic Science R&D Symposia at the American Academy of Forensic Sciences (AAFS) meetings. The abstract was not selected for an oral presentation in 2023 but the application was accepted for the 2024 conference in Denver, Colorado. In addition to professional presentations, workshops will be given to provide hands-on training to crime scene technicians, latent print, and pattern analysts on optimized protocols for the enhancement of blood impressions and semen smears from decedents in medicolegal cases. Student researchers have also gained valuable experience working on this project, many of which have now graduated and are now working in various aspects of the forensic profession. Several manuscripts are being prepared for publication on this project and student researchers will be co-authors on the submitted manuscripts. A manuscript is in preparation for publication in the Journal or Forensic Identification.

Acknowledgements

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VII. Conclusions

Discussion of Findings

Amido Black and Hungarian Red dye-stained blood impressions were best when visualized in situ on skin. The stained impressions, however, often lacked the desired contrast to visualize impression details, as the stain bonded to the blood but also absorbed into the semi-porous skin substrate creating background staining thus reducing visibility of impression details. Some of the Amido Black dye-stained impressions were able to be lifted from the skin using BVDA Gellifters® with partial impression patterns and some ridge path details visible on the white background of the lifter, but most of the lifted impressions had some proteinaceous materials with no ridge details

visible. Hungarian Red dye-stained impressions lifted with BVDA Gellifters® were not effective at the recovery of impressions and were unable to lift any visible proteinaceous material from the skin onto the black background of the lifter under both normal and alternate lighting. Even with the fluorescent properties of Hungarian Red their overall effectiveness was limited, and the resultant fluorescence was weak. BVDA Gellifters® were not effective in lifting the dye-stained impressions and impression details were clearly lost in transfer. Zar-Pro™ Fluorescent Lifters were highly effective in their ability to lift, enhance, and preserve blood and semen impressions from the skin of both the fetal pigs and human decedent donors. The data trends showed that as blood became more latent as a result of environmental factors and/or decomposition the recovered proteinaceous materials/impressions were not as readily visible on the Lifters under normal lighting conditions. As for the lifted semen smears, the latent nature of semen prevented visualization of the proteinaceous materials on the white background of the Lifters under normal lighting conditions. However, due to the fluorogenic properties of the Lifters, both blood and semen impressions were fluorescent and visible on the Lifters under alternate lighting conditions, with fluorescent intensity increasing as the Lifters dry as moisture from the activating solution will quench fluorescence.

The effectiveness of all three enhancement methods through the early stages of decomposition may result in a broader understanding of the ability to recover impression evidence from human skin in medico-legal cases. The recovery of aged, degraded, and/or damaged impressions from human decedent skin is possible, primarily until the epidermal skin is no longer intact, thus the threshold of impression recovery can be expanded through the later stages of early decomposition which was a meaningful part of this research project. Expanding forensic practitioner understanding of these aged, degraded limited, damaged, or otherwise compromised physical evidence, specifically the enhancement and recovery of blood impressions and semen smears from decedent skin during the early stages of decomposition is a worthwhile research endeavor. The ability to recover impressions across seasonal and geographical areas is another important aspect of this study and the recovery of impressions from frozen or snow-covered bodies or even extreme heat should not be dismissed.

Implications for policy and practice

Given the problems associated with methods currently used for the enhancement and preservation of impression evidence from decedents, the potential benefits of this research carry broad implications for criminal justice policy at all levels, from local to international. Improved guidelines will assist practitioners in the enhancement of visible proteinaceous impressions on decedent skin and increase awareness for the recovery of latent impressions. Resultant information has the potential to increase recovery of impression evidence from decedents in medicolegal cases, but also to provide guidelines for adoption into field protocols. Methodologies developed may contribute to the recoverability of impression evidence from human skin associated with violent crimes. A comparative assessment of the effectiveness of each enhancement method to include cost, feasibility, and ease of use for crime scenes will be generated and reported after field-testing conducted by the Wayne County Medical Examiners Officer (WCMEO). Most significantly, as a result of this project, a field manual outlining the protocol for the enhancement and preservation of proteinaceous impression evidence from decedent skin will be developed in conjunction with the WCMEO. The universal protocol will be reviewed for feasibility, then will be published, and made available for law enforcement entities in the country to use and or adopt to maximize the recovery of impression-based evidence. As stated in the 2004 Sampson and Sampson survey (51) only 1% of practitioners had attended training on the recovery of print evidence from human skin and only 12%

had attempted to process human bodies, whether alive or dead for impression evidence, concluding the field needs more "well-trained" and body processing should be attempted more often than it has been in the past. This study has demonstrated the tenacity of proteinaceous evidence on the skin of decedents, with this author being certain in the statement that if the epidermal skin is intact impression evidence may be available for recovery. Thus, changing the preconceived notion by practitioners working medico-legal cases that impression evidence is often not present when in reality there is a better chance that it is present on victim skin when associated with violent crimes, but it is just not being recovered. Improving practice associated with the recovery of this valuable evidence has the potential to directly influence standard practice in law enforcement improving the likelihood of the recovery of impression evidence from the skin of assault and homicide victims.

Implications for further research

The primary goal of this research project was to broaden the understanding of the detection and enhancement of impression evidence from human decedent skin during the early stages of decomposition. The seasonal variations in which the decedents were placed to decompose was not a primary focus of this project and could result in additional research opportunities. A continued focus on understanding environmental variations associated with the recovery of blood impressions and semen smears in different geographical locations, and weather conditions specifically precipitation which had the greatest impact on impression recovery in the research trials would be valuable in the medico-legal cases. In addition, efforts to improve macro photography in the field specifically when capturing impression evidence insitu on decedent skin should be explored. A big factor in the difficulty of capturing optimal quality impressions insitu on decedent skin in these trials was the time constraints associated with taking optimal quality impressions in field conditions. In addition, the contours of the body, skin itself has several substrate variables that lead to the difficulty of insitu photography which can be compounded by the artifacts associated with decomposition, such as insect activity on the epidermal surface and sub-epidermal maggot masses. Due to the number of impressions that were photographed at each collection interval the insitu impression quality was not optimal, reducing the number of impressions enhanced at each interval could be beneficial in future trials. The areas of future research as discussed would contribute much needed practical knowledge for the facilitation and implementation of the enhancement of proteinaceous impressions on decedent skin for medico-legal cases within the field of forensic science.

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