Vacuum Metal Deposition in combination with Aqua Leuco Crystal Violet, Hungarian Red or Amido Black

THESIS

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Preface

I'm presenting my research thesis, I wrote for my graduation internship. This internship was such a great opportunity to learn a lot more about forensic dactyloscopy. My knowledge has been broadened so much during this research. Writing this thesis was not possible without the help and support of friends, family, supervisor and teachers. Support and flexibility were very much needed in these weird times of the COVID-19 pandemic.

I would like to thank some people in particular. Starting with my supervisors from Loci Forensics B.V., Martin Eversdijk and René Gelderman. Thanks to their efforts, help and feedback this interesting thesis was written. I want to thank Bob Petersen, from the rural unit Zoetermeer for his help on Vacuum Metal Deposition. I also want to thank Erik de Groot, my supervisor from Van Hall Larenstein, for his guidance. And I want to thank Tristan Krap, my second supervisor from Van Hall Larenstein, for his good critics and feedback. In addition, I want to thank the assessors. Daan Burger, Hannah van Dieren, Esra Hoogeveen, Jacintha Knapen, Soeleka Mohamed, Melanie Need, Johannes Oldersma, Noah Teeling, Màrlene Többen and Roos van der Tol for scoring the fingerprints.

Hope you enjoy reading this thesis.

Christy Sari Putri Haurissa

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Samenvatting

Vingerafdrukken worden voornamelijk gecategoriseerd in drie vormen: patente afdrukken, latente afdrukken en vingerafdrukken in vervormbaar materiaal (driedimensionaal). Bepoederen is een veelgebruikte aanpak en werkt op veel verschillende oppervlaktes, behalve op statische ondergronden zoals een vuilniszak. Dit komt doordat de vingerafdruk dichtloopt wanneer deze bepoederd wordt. Dit komt door de statische elektriciteit van de vuilniszak. Een andere methode voor de visualisatie van vingerafdrukken is het opdampen van cyanoacrylaat. Een bijkomend nadeel van deze techniek is, dat het oppervlaktes die nat zijn geweest niet goed kan visualiseren. Wanneer stuk van overtuiging: een natte vuilniszak met combinatie sporen (latente vingerafdrukken en vingerafdrukken gezet met bloed), moet worden onderzocht, is vacuümmetaalafzetting (VMD) een passende techniek om de latente vingerafdrukken zichtbaar te maken. De methode berust op de afzetting van een dun laagje goud. Het goud wordt geabsorbeerd op de plaatsen waar vingerafdruk residu aanwezig is. Maar wordt niet geabsorbeerd op de plekken waar geen residu aanwezig is en blijft hier op het oppervlakte liggen. Na goud, wordt zink afgezet. Zink zal alleen hechten op plaatsen waar goud aan het oppervlakte zit. Hierdoor ontstaat er een visueel verschil op de plekken waar de vingerafdruk zat (gouden kleur) en de rest van de oppervlakte krijgt een zinken kleur (zilverachtig). Een andere moeilijkheid doet zich voor wanneer de helft van de afdrukken is gemaakt met bloed en de andere helft latent is. Soms is een afdruk zelfs, half latent en half gemaakt met bloed. Dan moeten bloedverbeteringstechnieken voor visualisatie van bloed worden uitgevoerd. Aqua Leuco Crystal Violet (ALCV), Hongaars rood en Amido Black zijn voorbeelden van deze bloedverbeteringstechnieken. Er is geen onderzoek gedaan naar de invloeden van VMD op een nabehandeling met ALCV, Hongaars rood en Amido Black.
De methode voor dit onderzoek was het maken van vingerafdrukken met bloed door drie donoren. Een deel van de monsters zijn gemaakt op twee objectglaasjes, daardoor stond de ene helft van een afdruk op het ene objectglaasje (hetzelfde voor de andere helft), zie figuur 8. Er werden twee verschillende tijdstippen genomen, 24 uur en twee weken (veertien dagen) voor de invloeden van ouderdom van de monsters. De helft van de monsters werd gevisualiseerd met VMD en gekleurd met ALCV, Amido Black of Hongaars rood. De andere helft van de monsters werd alleen gevisualiseerd met dezelfde bloedverbeteringstechniek. Alle monsters werden met elkaar vergeleken om te onderzoeken of er een statistisch significant verschil is. Ook werden verschillende oppervlakken, duct tape, filament tape en vuilniszak gebruikt.
Na visualisatie met behulp van VMD werden enkele latente vingerafdrukken zichtbaar, behalve bij filament tape. Alle latente afdrukken op filament tape waren enigszins zichtbaar na VMD. VMD laat een laag achter op de monsters, waardoor sommige vingerafdrukken gemaakt met bloed leken te glinsteren in het licht. Na behandeling met VMD werden, de met bloed gemaakte vingerafdrukken, zichtbaar gemaakt met ALCV, Amido Black en Hongaars Rood. De resultaten met betrekking tot ouderdom van monsters lieten geen significant verschil zien tussen beide tijdspunten. Ook lieten de resultaten tussen de drie verschillende bloedverbeteringstechnieken geen statistisch significant verschil zien. Er was wederom geen statistisch significant verschil te zien in de invloed van VMD, op ALCV, Amido Black en Hongaars Rood. Zelfs voor de vergelijkingen tussen de verschillende oppervlakten werd geen statistisch significant verschil waargenomen.
Wanneer er enig vermoeden is van combinatiesporen op een stuk van overtuiging is het zeer wenselijk om andere instellingen te gebruiken dan gebruikelijk is voor dat specifieke oppervlak. De focus ligt op de visualisatie van de latente vingerafdruk, niet op de patente afdrukken. Dit omdat VMD geen invloed heeft op bloed verbeteringstechnieken, ALCV, Amido Black en Hongaars Rood. De bloedverbeteringstechnieken spoelen de laag VMD weg, maar zullen de vingerafdrukken die met bloed zijn gemaakt zonder problemen visualiseren. De bevindingen in deze studie zijn erg belangrijk doordat ze nog niet eerder zijn gepubliceerd en zullen hopelijk worden erkend in verdere onderzoeken met combinatiesporen en VMD. Deze bevindingen zullen de volgordelijkheid veranderen waarin stuk van overtuiging wordt onderzocht.

Summary

Fingerprints are mainly categorized in three forms: patent, latent and plastic prints. There are many methods to recover a fingerprint depending on the type of print. Powdering is a widely used approach and works on many surfaces, except static surfaces such as a garbage bag. This is because the fingerprint clogs when it is powdered, due to the static electricity of the bag. Another method for the visualization of fingerprints is cyanoacrylate fuming. But with this technique an additional disadvantage is, that it cannot properly visualize on surfaces that have been wet.

When the piece of conviction is wet garbage bag, with combination traces of fingerprints, made with blood and latent fingerprints, VMD is an appropriate technique to reveal the latent fingerprints. The method relies on de deposition of a thin layer of gold, absorbed by the places where the fingerprint residue is present but will remain on the surface where there is no residue. After gold, zinc was deposited. Zinc will only attach on places where gold is on the surface. In that way the difference of the print and the metals are creating the visualization. Another difficulty comes around when, half of the prints are made with blood and half are latent. Some prints are even half latent and half bloody. Then blood enhancement techniques for blood visualization should be performed. Aqua Leuco Crystal Violet (ALCV), Hungarian Red and Amido Black are examples of these blood enhancement techniques. No study on the influences of VMD on ALCV, Hungarian Red and Amido Black has been published.

The method for this study was to make fingerprints with blood by three donors. Some samples were made on two microscopic slides, so one half of one print was on one microscopic slide (the same for the other half), see Figure 8. Two different time points were taken, 24 hours and two weeks (fourteen days) for the influences of anility. Half of the samples were visualized using VMD and stained using ALCV, Amido Black or Hungarian Red. And the other half of the samples were only stained using the same blood enhancement technique. All samples were compared to one another to see if there is a statistically significant difference. To investigate the influences of VMD on different surfaces, the following surfaces were used in this study: duct tape, filament tape and garbage bag.

After visualization of VMD, a few latent fingerprints were made visible with VMD, except for filament tape. All latent prints on filament tape were made slightly visible after VMD. VMD leaves a layer on the samples, making some fingerprints made with blood, shiny. After treated with VMD, the fingerprints made with blood were made visible using ALCV, Amido Black and Hungarian Red. The results of the anility of samples showed no significant difference between the two time points. The difference between the three blood enhancement techniques were also not statistically significant. The influences of VMD on ALCV, Amido Black and Hungarian Red were also not statistically significant. Even for the different surfaces, there was no statistically significant difference perceived.

When using VMD on combination traces it is very desirable to use different settings than normally used for that surface. The focus lays on visualization of the latent fingerprint, not the patent prints. Because VMD has no influence on ALCV, Amido Black and Hungarian Red. The blood enhancement techniques will wash away the layer of VMD. But will stain the fingerprints made with blood with no trouble. The findings in this study are very important because they have not been published yet. And hopefully will be acknowledged in further investigations using VMD and combination traces. These findings will change the sequence in which a piece of conviction will be investigated.

Index

1 Introduction

A fingerprint is a copy of the characteristic outward appearance of the fingertip epidermis. Or as Edmond Locard said, *"Every contact leaves a trace",* in this case a fingerprint. Johann Mayer made the first detailed description of the anatomical formation of fingerprints in 1788. He wrote that friction ridge skin is unique (Moenssens, 1971) (Cummins & Midlo, 1943). Sir William James Herschel was the first person to study persistence of friction ridges (Herschel, 1916). In 1880, Henry Faulds was first to publish in a scientific journal about the value of ridge skin for individualization. Faulds published in 1905 a guide to Finger-print identification (Faulds, 1905). In the first cases where fingerprints were used as evidence, the prints were manually indexed and matched. More and more cases came in, to be identified and the manual method became overwhelming. This led to the development of Automatic Fingerprint Identification Systems (AFIS), which is still used nowadays (Maltoni, Maio, Jain, & Prabhakar, 2009).

Fingerprints are mainly distinguishable in three forms. Patent prints (visible prints), latent prints, (not visible with naked eye) and plastic prints (formed in or on a substrate) (Langford, et al., 2005). Two (or more) prints can be matched by characteristic features, generally in three levels (Figure 1). Level 1 features are macro details or overall ridge patterns such as ridge flow and pattern type. Level 2 features refer to minutiae (Galton details) such as ridge bifurcations and endings. Level 3 features include all dimensional attributes of the ridge such as width, edge contour, pores, shape, scars and other permanent details (Mieloch, Munk, & Mihailescu, 2008).

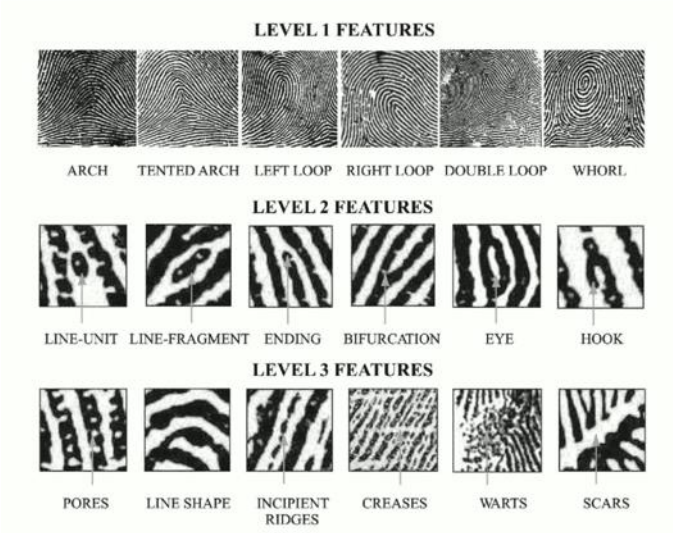


Figure 1: Three different levels features. Level 1 features are overall ridge patterns. Level 2 features are the minutiae. Level 3 features are all dimensional attributes (Mieloch, Munk, & Mihailescu, 2008).

Various methods and approaches can be used to recover a fingerprint depending on the type of print. The easiest approach to visualize latent fingerprints is powder dusting. The method relies on the mechanical adherence of the powder, to the sweat components of the skin deposits. Another method is chemical fuming. An example is cyanoacrylate (Superglue™) fuming. This method uses the deposition of polymerized cyanoacrylate ester onto the latent finger mark residue, to develop the print (Ramotowski, 2013). One downside of cyanoacrylate fuming is, that it cannot be used when a sample has been wet, in contrast to Vacuum Metal Deposition.

1.1 Vacuum Metal Deposition

Vacuum Metal Deposition (VMD) is used to develop latent prints. The method involves a thin coating or deposition of gold and zinc onto a surface, by evaporation using thermal sublimation under vacuum. The first one who applied this method was a French man, Theys et al (Theys, Turgis, Lepareux, Chevet, & Ceccaldi, 1968). They developed a fingerprint on paper by evaporation of a metal alloy. The method was being developed for a couple years. The first operational use of VMD was reported by Kent in collaboration with the Metropolitan police.

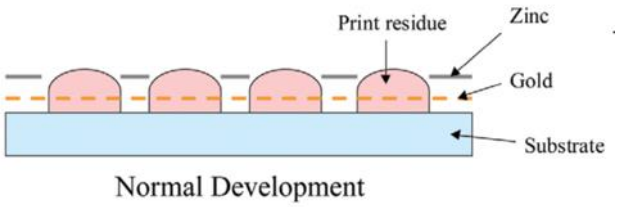


Figure 2: Schematic view of Vacuum Metal Deposition (National Institute of Justice, 2004)

VMD development works as follows: first a thin layer of gold is deposited. The gold diffuses on the fatty residues of the latent fingerprints, but will stay on the surface of places where there is no fatty residue. After the thin layer of gold, a layer of zinc is deposited. Zinc will attach on the places where gold is on the surface. Zinc will not attach on fatty residues (even if the residues are underneath the layer of gold), but it will deposit on small nuclei of metal, in this case gold (Figure 2) (National Institute of Justice, 2004) (Stroud, 1971). In this way creating a contrast between zinc and gold, which represent a visualisation of the fingerprint.

Both VMD and cyano fluming are effective methods of visualizing latent prints. But there is one difference, VMD has the advantage that, it can develop fingerprints on objects that have been wet or aged, whereas cyano fuming does not (National Institute of Justice, 2004). When investigating both methods on fabrics, VMD came out five times more likely to provide marks to include or exclude an individual (Fraser, Deacon, Bleay, & Bremner, 2014).

1.2 Staining solutions

Blood is known as one of the most common contaminants of fingerprints found at crime scenes (National Institute of Justice, 2004). Some fingerprints are (in)visible, some prints are underneath blood, and some prints are made in blood or combinations. In these combinations, parts of the fingerprint are latent, and parts are made with blood. To visualize fingerprints made with blood, blood enhancement techniques can be used. In this research, three techniques are used: Aqua Leuco Crystal Violet, Hungarian Red and Amido Black.

1.2.1 Aqua Leuco Crystal Violet

ALCV (Aqua Leuco Crystal Violet) is a colourless to pale blue aqueous solution. Leuco Crystal Violet is the completely reduced form of Crystal Violet. For the reaction to occur, hydrogen peroxide is needed. When ALCV and hydrogen peroxide comes into contact with the haemoglobin in blood, a catalytic reaction occurs, Figure 3. ALCV is oxidized by the hydrogen peroxide to the strong purple dye crystal violet, which gives the solution the purple/violet colour (Bodziak, 1995). The reagent can be used in two ways. It can be sprayed on the surface or immersed in the solution. If necessary, rinse with demineralized water, to rinse away unused reagent. Advantages are; in principle there is no need for rinsing after staining, it works on both porous and non-porous surfaces, the trace does not need to be fixed, the reagent already contains fixer (2% silver salicylic), it does not contain volatile solvents and is chemically safe (BVDA, 2020). Hemoglobin makes up roughly 95% of red cells' protein content and is made of four protein subunits, each containing a heme group. The heme group is made of a flat porphyrin ring and a conjugated ferrous ion.

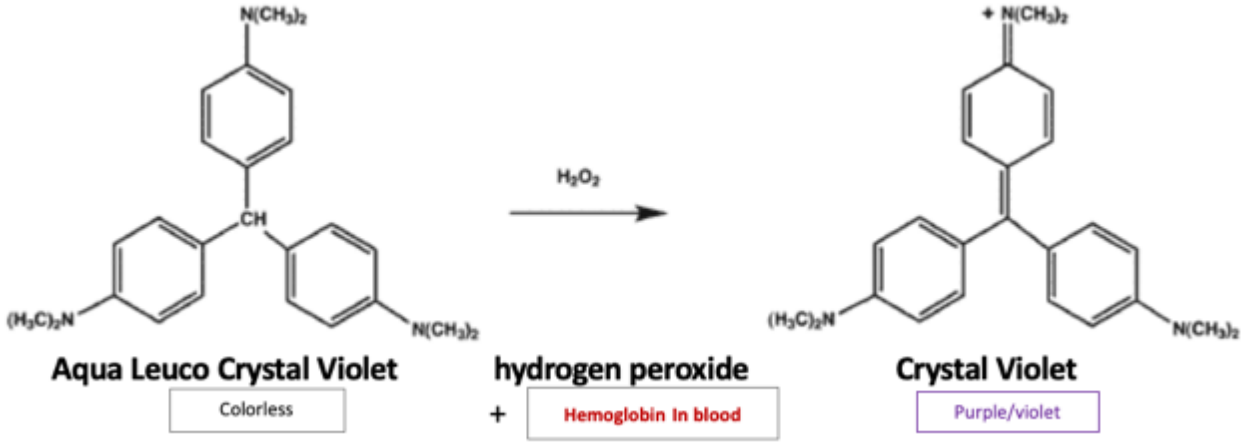


Figure 3: The catalytic reaction of ALCV with hydrogen peroxide and the hemoglobin in blood to crystal violet. ALCV was colorless and will turn into a purple/violet solution after the catalytic reaction (BVDA, 2020).

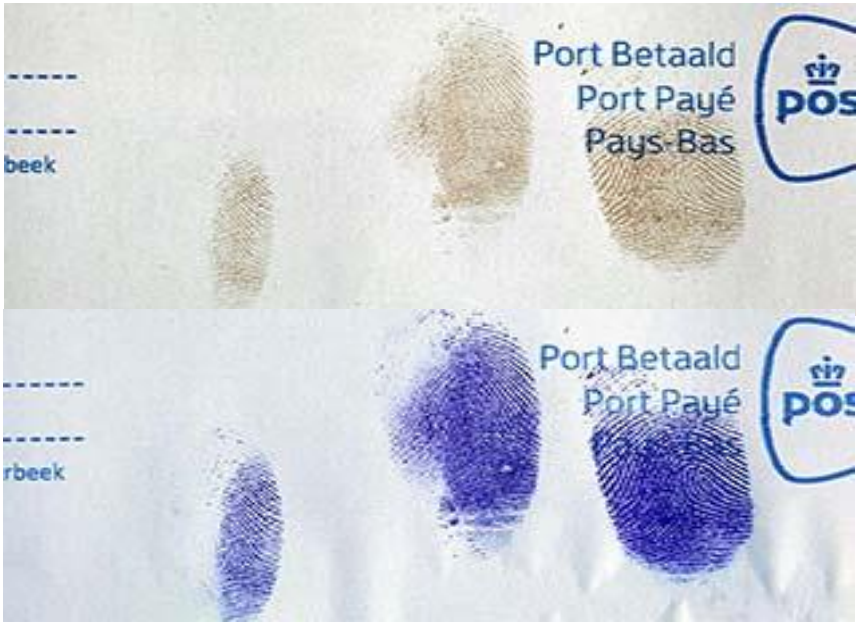


Figure 4: Example of fingerprints stained with ALCV (BVDA, 2020).

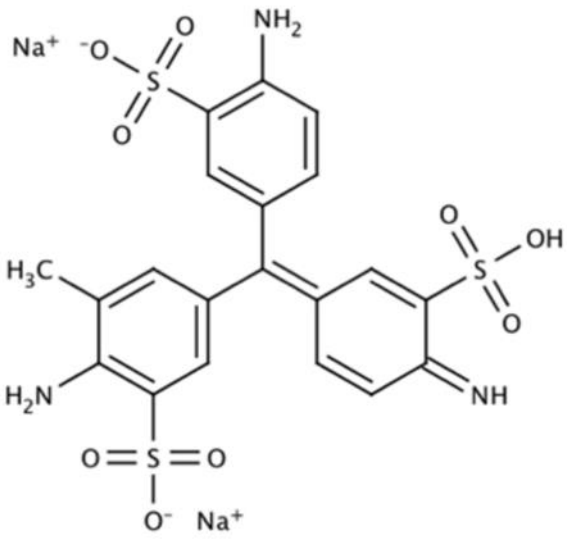


Figure 5: Structural formula of Hungarian Red, Acid Fuchsin, Acid Violet 19 (BVDA, 2020).

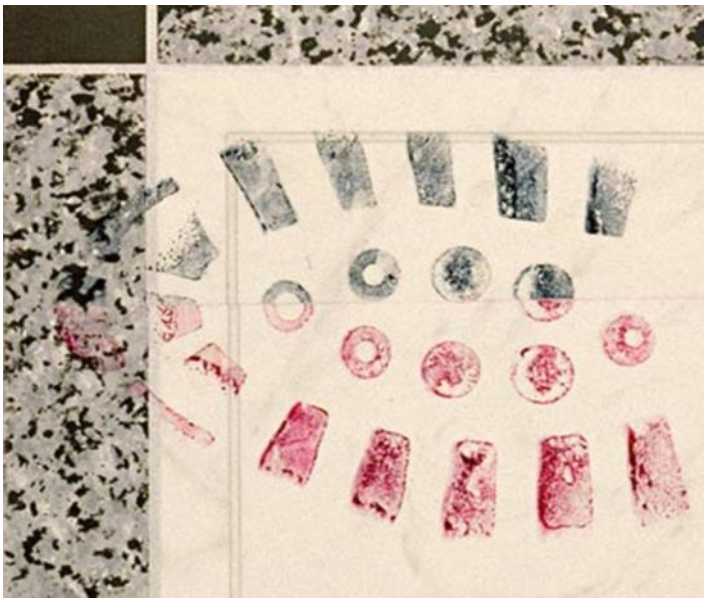


Figure 6: A shoe print stained with Amido Black (upper half) and Hungarian Red (lower half) (BVDA, 2020).

1.2.2 Hungarian Red (Fuchsin Red)

Hungarian Red or Fuchsin Red is a water-based dye, used to visualize (traces of) blood. It is a safe agent because it is water based and already contains the fixation of 2% silver salicylic. When the reagent comes in contact with the protein in blood, it will give a bright red colour. Hungarian Red is not suitable for absorbent surfaces and the solution will start to flow after some time. That is why, it is important to capture details as soon as possible (BVDA, 2020). A great advantage of Hungarian Red is, that the prints can be lifted with white gelatine foil. The lifts can be very useful when working with curved or dark surfaces. Another great advantage is, that the lifted prints will fluoresce when illuminated with green light (515-560 nm) and a red filter.

1.2.3 Amido Black

Amido Black or Acid Black 1 has been long known for staining proteins (Kent, 2004). It will bind to proteins present in blood, and give a black to blue stain. Amido Black will not detect normal eccrine and/or sweat components of fingerprints. Therefore, it is used when it is believed that the fingerprint consists to the most extend of blood (Kent, 2004). Amido Black is supplied on a methanol basis, as well as an aqueous solution. Amido Black in methanol has a stronger colour but is also more dangerous, due to the toxicity of the methanol (BVDA, 2020).

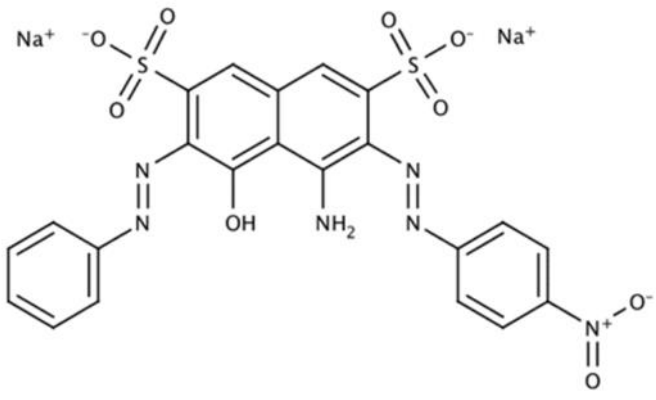


Figure 7: Structural formula of Amido Black/Acid black 1. Source: BVDA.

Blood enhancement techniques fall mainly into two types: Those that use the heme group to prove the presence of blood and those that react with proteins or their breakdown products (Sears, Butcher, & Fitzgerald, 2005). . Hungarian Red and Amido Black are both staining proteins in blood. Whereas ALCV has the characteristic that, it uses a REDOX reaction to stain hemoglobin in blood. Hungarian Red has the advantage that it can be lifted using a white gelatin lifter and in this way, easy to use on dark or curved surfaces. One disadvantage is that Hungarian Red has to be captured as soon as possible, because the staining will eventually flow and the details of the fingerprint will fade. Amido black has the advantage that it does not flow and no rush to be captured. But it cannot be lifted as good as Hungarian Red.

1.3 Issue

Following case was in the Dutch papers: As a statement in the criminal circuit to an enemy, a severed head of a victim was placed in a garbage bag on the street in front of a shisha lounge in Amsterdam Southeast. The bag was found on an early morning in March 2016 with a temperature of ±7.5 °C. On the outside of the bag dew condensation was formed, making the bag wet on the outside. Obviously, the garbage bag will contain a lot of blood, but also perpetrator traces such as fingerprints.

In the fictional case above some main problems arise concerning fingerprint recovery:

- The elapsed time from making the print to discovering the print
- The nature of the surface on which the print is present
- The method(s) to recover, sequencing.

When an object has been wet due to any kind of factor, cyano fuming is not possible anymore. VMD is an alternative to cyano fuming. But VMD can have influences on bloodstaining methods, which are not investigated yet. This presumption can be explained by the fact that, VMD treatment leaves a layer of gold and zinc on the surface of an sample. These metals may interfere with the blood enhancement techniques. Because Hungarian Red and Amido Black have a direct ionic interaction between the acid groups of the dyes, with the basic groups of the proteins (Schmidt, 1938). Zinc and gold may disturb these ionic interactions. ALCV has a REDOX reaction under the influence of hydrogen peroxide, with the hemoglobin in blood. A layer of metals may disturb this reaction, because the charge of metal clusters may change during a catalytic cycle (Lui & Corma, 2018). This issue can be examined by the main question: Is it possible to use Aqua Leuco Crystal Violet, Hungarian Red or Amido Black, after VMD development?

Following sub-questions arise with the main question:
Post VMD

- Is it necessary to use a blood enhancement technique after VMD?
- Is there a difference in visualisation between the three blood enhancement techniques, after VMD?
    - Is there a difference in visualisation between protein dyes and hemoglobin dye?
    - Is it still possible to lift a fingerprint stained with Hungarian Red, after VMD?
- Is there a statistically significant difference ($p<0.05$) in the visualisation of older fingerprints ?

VMD vs. No VMD

- Is there a statistically significant difference ($p<0.05$) in samples treated with VMD, vs. samples without VMD?
- Is there a statistically significant difference ($p<0.05$) between different surfaces?
- Is there a difference in detection limit of blood of the blood enhancement techniques, in samples treated with VMD vs. samples without VMD?

2 Approach

To investigate if it is still possible to use Aqua Leuco Crystal Violet, Hungarian Red or Amido Black, after VMD development, the following approach was executed as a result of Appendix VI – Plan of approach. The three bloodstaining methods used in this study were; Hungarian Red, Amido Black and ALCV. Different microscopic slides were provided with a fingerprint by three different donors, treated with VMD by the rural unit of Zoetermeer and lastly stained with a blood enhancement technique. Another batch had the same method of fingerprint sampling but was not treated with VMD. These two groups were scored by ten different assessors using scoring table by Almog et al. These scores were compared to one another other using a Mann-Withney U test.

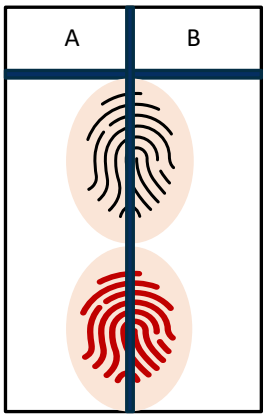


Figure 8: Two object slides (A and B) with 2 fingerprints. The blue print is a regular fingerprint. Red print is a fingerprint made with blood.

2.1 Samples

The fingerprints were provided by three different donors. The donors used in this study were a mix of males and female, ranged in age (twenty-two to fifty-five years old). Prior to collecting the fingerprints, the donors did not wash their hands for at least one hour. To create the best duplo's, one fingerprint was put onto two slides at the same time. In this way, one sample contained one halve of a print. And another sample contained the other halve of the print. One sample contained two fingerprints: one latent fingerprint and one fingerprint made with blood (Figure 8).

2.2 Anility

VMD is known for good visualization of old fingerprints, up to 24 months old (Masters & DeHaan, 1996). For that reason, a small timeframe was used, to investigate if this characteristic was still applicable in combination with ALCV, Hungarian Red and/or Amido Black. Two groups of samples were prepared. One group was prepared 24 hours before examination. And another group was prepared two weeks (fourteen days) before examination. The samples were kept away from sunlight, at 60% humidity and 20°C

2.3 Dilutions

Different blood dilutions were put onto one microscopic slide. These microscopic slides contain three dimples. In this way the dilutions were made in triplo. 25 µl of dilution was put into one dimple. 9 samples will be made for each concentration of 1:100, 1:500 and 1:1000, 27 samples in total. For the negative control, water was used. In Figure 9, an example of a microscopic slide is shown. The samples will be dried for at least 24 hours, kept away from sunlight, at 60% humidity and 20°C.

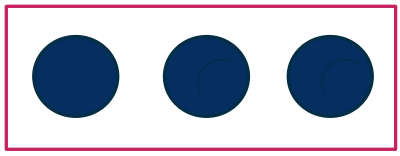


Figure 9: A microscopic slide with 3 dimples. In every dimple the same dilution of blood is added. In this way the samples are made in triplo.

2.4 Surfaces

For the research on different surfaces, the following surfaces were used: garbage bag, duct tape, filament tape and aluminium. For each surface 24 samples were taken.

2.5 VMD

VMD treatment of the samples was carried out by the rural unit Zoetermeer, using an Oerlikon Leybold Univex 450. All microscopic slides were put onto a plate and put into the VMD hood (Figure 10). The samples were developed following the instructions of the manufacturer.

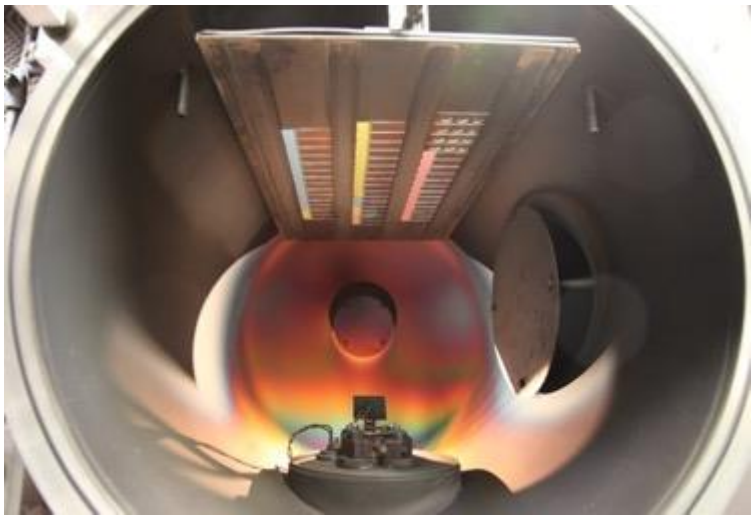


Figure 10: Inside of the Oerlikon Leybold Univex 450 VMD with the microscopic slide samples inside.

Figure 11: Oerlikon Leybold Univex 450, used by the rural unit of Zoetermeer

The equipment of VMD consists of, a vacuum chamber, filaments for evaporation of gold and zinc and a window to monitor the deposition of the metals (Figure 11). Gold deposition takes place when the pressure in the chamber has reached 3 x 10^{-4} mbar or lower, and the current to the filament is increased until the filament reaches a yellow-to-white heat. The deposition of gold should be complete within 10 seconds. But, when not all gold has been evaporated, the temperature should be increased until all residue is gone. After the gold deposition, the pressure in the camber is increased and the current to the zinc deposition is turned on. Increasing the pressure in the camber is to reduce the speed of zinc deposition by introducing more air molecules with which the zinc may collide. Zinc deposition filaments are larger and deeper than the gold filament, and the quantity of zinc added is greater. For zinc deposition, the current is increased until the filament glows a cherry-red to dull orange color. This process should be observed by the operator, trough the viewing window. After zinc deposition, the gold filaments should be heated shortly to yellow-to white heat to burn off zinc contamination (Kent, Manual of Fingerprint Development Techniques, 2004) (National Institute of Justice, 2004). The average pressure was 1,7 x 10^{-4} mbar. For each different surface, the time of deposition will differ. Therefore, it is important to examine each surface individually in the chamber. All samples were treated in a minimum of six different batches.

2.6 Blood enhancement techniques

All samples were equally divided and developed using one of the three blood enhancement techniques. The solution for ALCV contains the fixation for blood, but needs to be activated by 3% hydrogen peroxide. The solution could be applied directly to the samples. The application was done by immersion of the samples. Immersing took place in homogeneous solutions, using a stirring flow. Places where ALCV solution makes contact with blood, the blood impression turns a purple to violet color. The reaction takes place fast, so immersing only took place for about 10 seconds. After one minute of reacting, if necessary, the samples were immersed again. When the fingerprint was made visible as desired, the sample was immersed in the cleaning liquid (0,01% natural vinegar).

For Hungarian Red and Amido Black, the solutions contain the fixation for blood too. The application was also done by immersion of the samples. For these solutions, the immersion of the samples needs

to be done for four minutes per sample. After immersion in the staining solution, the samples were immersed in the cleaning liquid (0,01% natural vinegar) (NC Office of Indigent Defense Services, 2012).

2.7 Scoring

Ten different assessors, all forensic science students, scored the fingerprints following Table 1. This scaling table was made by Centre for Applied Science & Technology (CAST) (Almog, Cantu, Champod, Kent, & Lennard, 2014). The assessors gave the fingerprints a score from 0 – 4.

Table 1: Grading system for the assessment of fingermark detection techniques International Fingerprint Research Group (IFRG) (Almog, Cantu, Champod, Kent, & Lennard, 2014).

Grade	Details
0	No development
1	Signs of contact but < 1/3 of mark with continuous ridges
2	1/3–2/3 of mark with continuous ridges
3	> 2/3 of mark with continuous ridges, but not quite a perfect mark
4	Full development – whole mark clear with continuous ridges

2.8 Validation

Prior to using assessment tools for research, the reliability must be established (Koo & Li, 2016). Intraclass correlation coefficient (ICC) is a commonly used reliability index. An ICC-test will be performed on all data to determine, whether the different assessors agree with one another, and whether the data to be used is reliable (Bruton, Conway, & Holgate, 2000). Since the fingerprints were placed in the center of two microscopic slides, it can be reasonably assumed that the two halves of one print have more similarities than two random halves. For this reason, a paired test was used.
Mann-Whitney U test was used, since the data was assumed to be not normally distributed. This assumption was made because a shift from the mean was expected.

3 Results

The aim of this study was, to investigate the effects on VMD on blood enhancement techniques. This was done by comparing samples that were treated with VMD, to samples that were not treated with VMD. Both treated with blood enhancement techniques. Scores were given by ten different assessors, using the scoring model of Almog et al. (Almog, Cantu, Champod, Kent, & Lennard, 2014). To examine to which extend the assessors agreed with one another Intraclass correlation was carried out, two way mixed and with absolute agreement.

Table 2: ICC of the different sample groups. With the 95% confidence interval and the agreement. VMD, no VMD and surfaces no VMD had a good agreement. And surfaces VMD had an excellent agreement.

Samples	Intraclass Correlation	95% Confidence Interval	Agreement
VMD	0.891	0.840 – 0.928	Good
No VMD	0.725	0.535 – 0.846	Good
Surfaces VMD	0.946	0.921 – 0.964	Excellent
Surfaces no VMD	0.876	0.795 – 0.927	Good

3.1 Post VMD

After the samples were treated using VMD, some of the latent fingerprints were visible. In contrast to the fingerprints made with blood, almost all fingerprints were made visible only using VMD. Figure 12 shows an example, four fingerprints made by two different donors on microscopic slides. The top fingerprints were the latent prints. The top left fingerprint is visible, but the top right is not developed. Whereas for the two bottom prints, both prints are made visible quite good, after only VMD. All latent prints on the batch with filament tape were slightly made visible after VMD. The fingerprints made with blood on duct tape, filament tape and on the garbage bag showed shimmering in the fingerprint (Figure 13). Treatment with VMD alone did not show enough detail to identify the fingerprints.

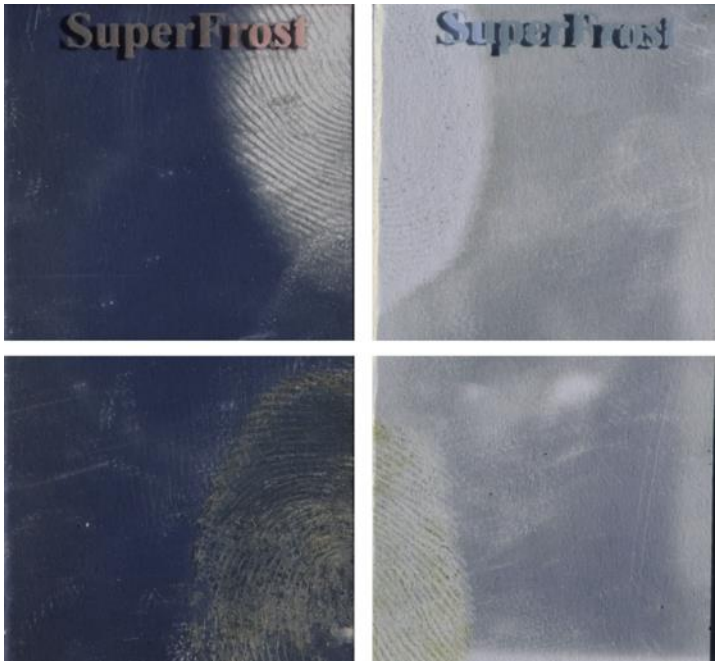


Figure 12: Samples on microscopic slides after VMD. Top left and right, latent fingerprint. Bottom left and right, fingerprint made with blood.

Figure 13: Fingerprint made with blood on a garbage bag after VMD. The fingerprint is very shiny/shimmering.

All prints were equally divided over the three blood enhancement techniques. For the microscopic slides one print (two halves) was divided between two different blood enhancement techniques (Figure 14).

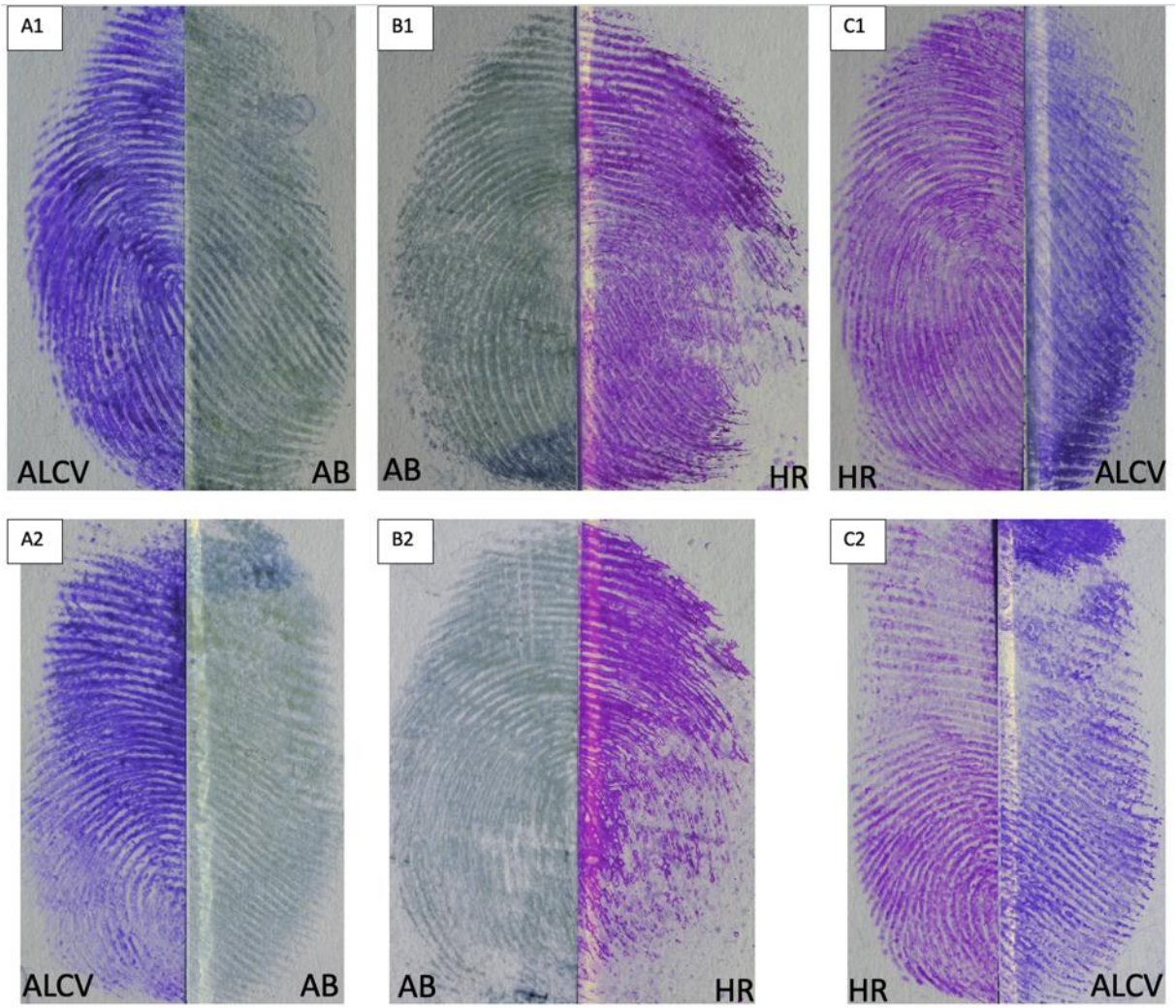


Figure 14: ALCV: Stained with ALCV, AB: stained with Amido Black, HR: stained with Hungarian Red. The fingerprints are two halves of one print and each print is treated with another blood enhancement technique. A1, B1 and C1 were treated with VMD and stained with a blood enhancement technique afterwards. A2, B2, C2 were only visualized with a blood enhancement technique.

3.2 Influences of VMD

To investigate the influences of VMD on ALCV, Amido Black and Hungarian Red, samples treated with VMD and without VMD were compared per blood enhancement techniques. The samples contained a latent fingerprint and a print made with blood. Following Mann-Whitney U test were carried out to show the statistically significant difference:

- ALCV with VMD & ALCV no VMD
- Amido Black with VMD & Amido Black no VMD
- Hungarian Red with VMD & Amido Black no VMD

Table 3: Results of Mann-Whitney U test of the difference between VMD and no VMD on ALCV, Amido Black and Hungarian Red. All groups had no statistically significant difference.

Group	Asymptotic Significance	Stat. sig. difference (p <0.05)
ALCV VMD	0.608	No statistically significant difference
ALCV no VMD		
Amido Black	0.655	No statistically significant difference

Amido Black no VMD		
Hungarian Red VMD	0.728	No statistically significant difference
Hungarian Red no VMD		

For ALCV treated with and without VMD, the asymptotic significance was 0.882. Meaning there was no statistically significant difference. Amido Black had an asymptotic significance of 0.986, also no significant difference. For the last blood enhancement technique, Hungarian Red, also no significant difference was seen, because the asymptotic significance was 0.804.

3.3 Differences blood enhancement techniques

Since there was no statistically significant difference between treatment with VMD and without. The three blood enhancement techniques were compared to one another to see if there were any differences between ALCV, Amido Black and Hungarian Red. Mann-Whitney U test was executed in the following way:

- ALCV & Amido Black
- Amido Black & Hungarian Red
- Hungarian Red & ALCV

Table 4: Results of Mann-Whitney U test of ALCV, Amido Black and Hungarian Red. All groups had no statistically significant difference.

Group	Asymptotic Significance	Stat. sig. difference (p <0.05)
ALCV	0.731	No statistically significant difference
Amido Black		
Amido Black	1,00	No statistically significant difference
Hungarian Red		
Hungarian Red	0.773	No statistically significant difference
ALCV		

Table 4 shows the comparison of the three blood enhancement techniques on one another. There was no statistically difference found between any blood enhance technique.

3.4 Anility

For the anility, fingerprints with and without blood were taken at two different times. One group was taken 24 hours before examination and another group was taken two weeks before examination. Following Mann-Whitney U test were carried out to examine if there is a statistically significant difference between the two points of time.

- ALCV two weeks & ALCV 24 hours
- Amido Black two weeks & Amido 24 hours
- Hungarian Red two weeks & Amido Black 24 hours

Table 5: Results of Mann-Whitney U test of the anility of ALCV, Amido Black and Hungarian Red. All groups had no statistically significant difference.

Group	Asymptotic Significance	Stat. sig. difference (p <0.05)
ALCV 2 weeks	0.932	No statistically significant difference
ALCV 24 hours		

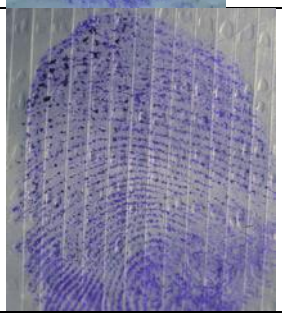
Amido Black 2 weeks	0.514	No statistically significant difference
Amido Black 24 hours		
Hungarian Red 2 weeks	0.242	No statistically significant difference
Hungarian Red 24 hours		

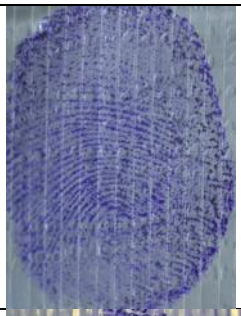
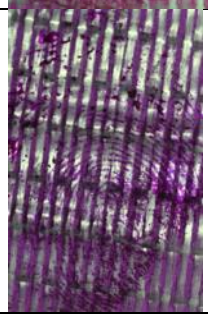
Table 5 shows the results of the Mann-Whitney U test. When the asymptotic significance is below 0.05 the difference is statistically significant. The asymptotic significance for ALCV was 0.799, for Amido Black it was 0.410 and Hungarian Red was 0.590. For all three blood enhancement techniques there was no statistically significant difference between two weeks and 24 hours.

3.5 Surfaces

Table 6 shows an overview of a few samples of different surfaces and different blood enhancement techniques, treated with or without VMD. Overall, no distinct differences were observed, except for filament tape. With the surface of filament tape, it was hard to photograph the fingerprint. Especially for Hungarian Red on filament tape.

Table 6: Overview of samples with different surfaces, with or without VMD and after a blood enhancement technique (ALCV, Amido Black or Hungarian Red.

	ALCV	**Amido Black**	**Hungarian Red**
Garbage bag VMD			
Garbage bag no VMD			
Duct tape VMD			

Duct tape no VMD			
Filament tape VMD			
Filament tape no VMD			

One thing was a remarkable result. On all filament tape samples that were visualized using VMD, the latent print was made visible on all samples. This could be explained by the difference in plastic substrate of filament tape, to the other surfaces. After staining with ALCV, the latent prints were not washed away like in the other surfaces but were stained by ALCV (Figure 15). Not as clear as the staining on the bloody fingerprints, but more visible than on all other samples.

Three different surfaces contained a latent fingerprint and a fingerprint made with blood. The fingerprints made with blood were stained with different blood enhancement techniques and compared to one another. In this way, investigating the differences between samples, treated with and without VMD, on these surfaces. Following Mann-Whitney U tests were executed:

- Garbage bag VMD & garbage bag no VMD
- Duct tape VMD & Duct tape no VMD
- Filament tape VMD & filament tape no VMD

Table 7: Results of Mann-Whitney U test different surfaces using ALCV, Amido Black and Hungarian Red. All groups had no statistically significant difference.

Group	Asymptotic Significance	Stat. sig. difference (p <0.05)
Garbage bag VMD	0.667	No statistically significant difference
Garbage bag no VMD		
Duct tape VMD	0.179	No statistically significant difference
Duct tape no VMD		
Filament tape VMD	0.198	No statistically significant difference
Filament tape no VMD		

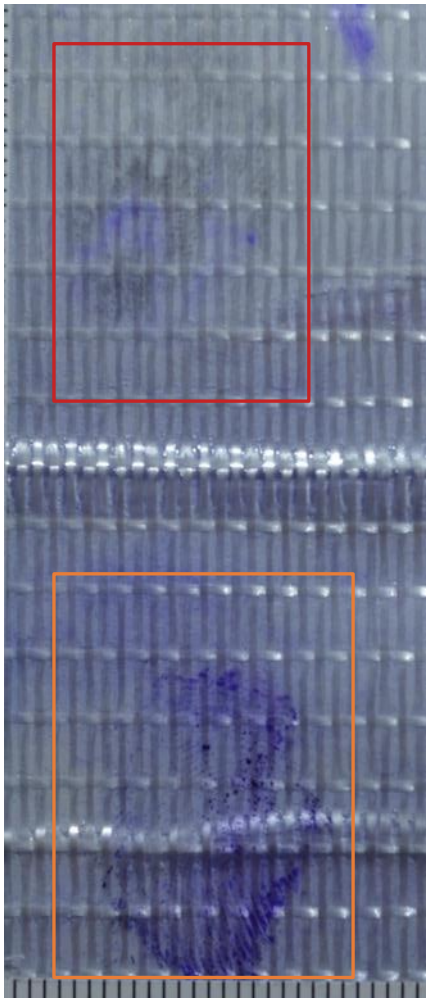


Figure 16: Sample of armored duct tape after VMD and visualized using ALCV. Orange rectangle is a fingerprint made with blood. Red rectangle is a latent fingerprint. There are some VMD residue visible in the red rectangle, and some of the VMD residue is stained by ALCV.

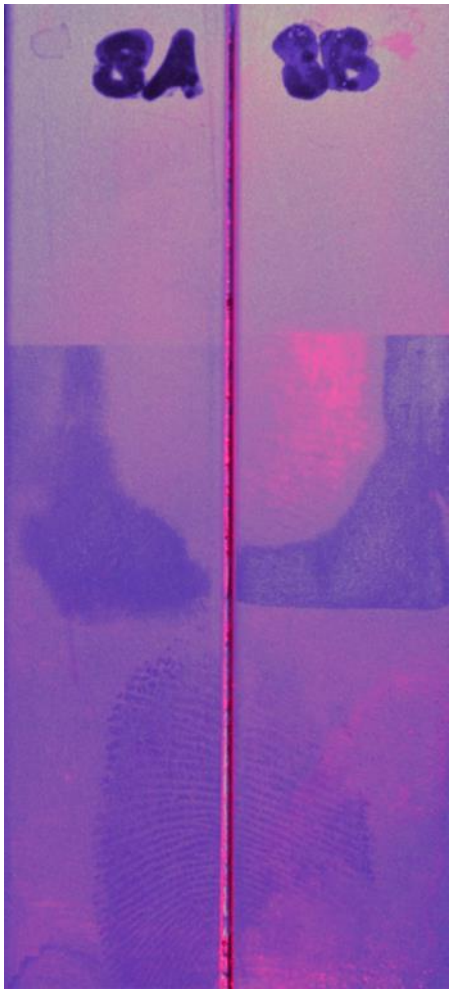


Figure 15: Sample of aluminium. Upper fingerprint is the latent fingerprint, powdered using fluorescent powder. Lower fingerprints are made with blood. Lower left half is stained with Hungarian Red and lower right half is stained using ALCV.

3.5.1 Aluminium

As described in the approach, aluminium was initially taken into account in this research. The fingerprints, made with blood, on aluminium were treated with VMD, visualized with a blood enhancement technique and also scored. But when the samples without VMD had do be made, the specific aluminium was not available anymore. For that reason, aluminium was not further used for comparison to other surfaces in this study. To not waste the aluminium samples, there was a test done with a fluorescent powdering technique on the latent fingerprint. The latent fingerprint, after treated with VMD, was not treated with any blood enhancement technique. The fluorescent powder gave some result but not as clear as the fingerprint made with blood and enhance with a blood enhancement technique (figure 16).

3.6 Dilutions

For the detection limit of ALCV, Amido Black and Hungarian Red after VMD, different microscopic slides were stained with the blood enhancement techniques. De dilutions were 1:100, 1:500 and 1:1000. For all three blood enhancement techniques the results were the same. The detection limit lies between the concentration of 1:500 and 1:1000 of blood.

4 Discussion

The aim of this study was to investigate the possibility, to use Aqua Leuco Crystal Violet, Hungarian Red or Amido Black, after VMD development. VMD is an effective method and continues where cyanoacrylate is limited (National Institute of Justice, 2004). Hungarian Red and Amido Black are staining solution that will stain protein in blood. The basic mechanism of Hungarian Red and Amido Black reactions with protein, would be a direct ionic interaction between the acid groups of the dye with the basic groups of the protein (Schmidt, 1938). The layer of gold and zinc, VMD gives the samples, may disturb this ionic interaction. That is why was expected that VMD has a negative influence on Amido Black and Hungarian red. For ALCV, the expectations were the same. Because the REDOX reaction ALCV has, with the hemoglobin in blood, the metals from VMD might influence the reaction. For the reason that the charge of metal clusters may change during catalytic cycle, when REDOX reaction take place (Lui & Corma, 2018). These influences let it come to the hypothesis: VMD might have a negative influence on ALCV, Amido Black and Hungarian red.

Overall, 255 samples (fingerprints made with blood), were taken made by three different donors. The donors were males and female and raged in age. These donors had experience with placing fingerprints with blood for other previous studies. All samples have been scored 0 – 4, by ten different assessors. These scores were compared to one another and an ICC was calculated. The results of the ICC were good to excellent, meaning that there was a good agreement between the assessors. The assumption was made that the data would be not normally distributed. When two groups have a statistically difference, it is not possible for the data to be normally distributed (Bruton, Conway, & Holgate, 2000). For that reason, Mann-Whitney U test was chosen over a t-test. For a t-test the data has to be normally distributed, and a shift in data is not possible. The data in this study is continues data, and in that way does not meet the requirement of Cohen's Kappa. For that reason, there was no Inter- or Intra-observer reliability calculated.

All samples were divided into two main groups. One group was treated with VMD, and the other group was not. For the group that was treated with VMD, post VMD, not all samples came with a good result on the latent print. The samples were treated with VMD in a minimum of six different batches. Each batch only contained one type of surface (microscopic slides, duct tape, filament tape, aluminium or garbage bag). In this way the difference, in which the gold and zinc react to the surface, is equal to the whole batch. The VMD was executed by another party, meaning that the exact settings for each batch were not clear. Even which microscopic slides belongs to which batch was not clear. But something that was clear, was that the settings used were not appropriate for the development of the latent prints. Post VMD, the fingerprints made with blood were made visible with the naked eye. But were not that detailed to identify the fingerprint. On some surfaces, the fingerprint made with blood even seem to shimmer, making it hard to capture minor details. Meaning that, it was still necessary to use a blood enhancement technique after VMD.

All blood enhancement techniques that were used in this study, contained the fixation for blood. In this way the solutions were water based and in the eyes of durability better for people as well as for planet. The method for staining is also more durable, immersing in the solution uses less liquid than spraying 255 samples. Which has a positive effect on the profit side of this study.

During the first phase of the study, the influences of VMD on ALCV, Amido Black and Hungarian Red were examined. The differences of VMD on ALCV were not statistically significant. There were some doubts in the expectation of ALCV and VMD together. Because the catalytic reaction, could interfere with the metals of VMD. When redox reactions take place, the charge of metal clusters may change during catalytic cycles (Lui & Corma, 2018). For Amido Black and Hungarian Red there was also no significant difference found. An explanation for this, is the observation that all staining solutions "washed" away the VMD layer. The fingerprint, made with blood, was clearly made visible after any

blood enhancement technique. No difference was observed for any sample, in the quality of the fingerprints, with or without VMD. In contrast to the few latent prints that were visible after VMD. All of the latent prints were not visible anymore after the blood enhancement techniques. This was the same for all samples on the microscopic slides, and each blood enhancement technique: ALCV, Amido Black and Hungarian Red.
In the second phase, the blood enhancement techniques were compared to one another, to investigate if one of the three was better. For ALCV, Amido Black and Hungarian Red the outcomes were the same, no statistical difference was found. This does meet up with the expectation, because all three blood enhancement techniques are from the same quality. The only differences between ALCV, Amido Black and Hungarian Red are: the color of the staining solution, protein or hemoglobin staining, and liftable or not. So, no differences in the intensity of the staining.

In general, VMD is more sensitive than other latent fingerprint development techniques. VMD even has greater sensitivity on older finger marks (Masters & DeHaan, 1996) (Misner, 1992). To investigate if these features will still be applicable, in combination with the blood enhancement techniques, a difference in time points was taken into account in this study. Samples were taken at two time points, 24 hours before examination or two weeks (fourteen days) before examination. These samples were treated with VMD and stained with a blood enhancement technique afterwards. For VMD, Amido Black and Hungarian Red, there was no statistically significant result between the two time points.

Different surfaces were investigated, with and without VMD treatment. For the samples made on duct tape and garbage bags, none of the latent prints were made visible after only VMD. In contrast to the filament tape samples, where all latent prints were (slightly) visible. After the blood enhancement techniques, there were no statistically significant difference found between duct tape, filament tape and garbage bag. One remarkable result was that with all filament tape, latent prints were made visible after only using VMD. Meaning that, the settings of the VMD, for the filament tape batch was right. Staining these filament tape samples after VMD, using ALCV gave a remarkable result. Parts of the latent print actually was stained as well. This cannot be explained by the fact that, there were some residues of blood on the latent fingerprints. This is because it was the case with all donors. Thereby, the donors first made the latent prints, and afterwards the prints with blood. The results on the latent fingerprints on filament tape, might be explained by the differences in surfaces. Garbage bags are made out of LDPE (Low-density polyethylene). Duct tape is a textile-based adhesive tape and is coated with a polyethene layer, which makes the tape waterproof. Filament tape is stronger than duct tape. Filament tape is made out of polypropylene with woven cotton strings. Jones et al. did two studies on the influences of polymer type on VMD (Jones, Stoilovic, Lennard, & Roux, 2001) (Jones, Mansour, Stoilovic, Lennard, & Roux, 2001). These studies confirm that VMD development is affected by polymer type and even shows differences in VMD development within one polymer type. This can be explained by the fact that all plastics contain additives (e.g., plasticizers or dyes) (Zhang, Shen, & Somorjai, 1992). These additives might change the surface characteristic of the plastic, and in this way affect VMD development.
Aluminium was initially one of the different surfaces that would be investigated. Due to circumstances, the aluminium used, was not available anymore for comparison. But the aluminium samples were still stained with the different blood enhancement techniques. Resulting in good and very clear developed fingerprints. Due to this good result, further research it is recommended using aluminium as a surface. Another difficulty was photographing the filament tape, stained with Hungarian Red. This solution tends to run out over time and stained the background of the tape pink too. Thereby, the surface has a woven thread and reflects the light. It is possible and useful to take a lift with white gelatin lifter, after staining with Hungarian Red (BVDA, 2020). All the samples of filament tape were lifted, after leaving the lift on for the minimum amount of time necessary (forty minutes). For both the samples treated with, as without VMD, the lifts were not a good representation of the print. As a solution for this problem, it is recommended leaving the gelatin lifter on for at least one hour (sixty minutes).

The last phase was to investigate if VMD had influence on the detection limit of ALCV, Amido Black or Hungarian Red. The samples treated after VMD, had a detection limit between 1:500 and 1:1000. This detection limit was the same for all three blood enhancement techniques. The same detection limit between 1:500 and 1:1000, was observed for samples that were not treated with VMD. This did not meet the expectations, because it was expected that VMD would have a disturbing influence on any blood enhancement technique.

5 Conclusion/recommendations

The aim of this study was to investigate the possibility, to use Aqua Leuco Crystal Violet, Hungarian Red or Amido Black, after VMD development. When using VMD, it is very desirable to use different batches for different surfaces. And is very beneficial to determine the polymer type before VMD treatment. The polymer type can be easily determined using Fourier transform infrared spectroscopy (FT-IR) (Suzuki, 1993). With this information on polymer type, different gold concentration should be used (Jones, Mansour, Stoilovic, Lennard, & Roux, 2001). Another important fact to keep in mind, if the samples (potentially) have latent fingerprints as well as, fingerprints made with blood (combination traces). These combination traces may need different settings using VMD than normally used with such surface. The focus lays on visualization of the latent fingerprint. When the latent print is made visible, photographing and documentation of the print is desired before treating the print with blood enhancement technique. The blood enhancement technique will wash away the layer of VMD, but will stain the fingerprints made with blood with no trouble.

Regarding the main question, there were no statistically significant differences found between the samples developed with VMD and without. Regarding the different blood enhancement techniques, there was no statistically difference between ALCV, Amido Black and Hungarian Red. When looking at anility of the samples, there was no statistically difference found between anility of two weeks or 24 hours for ALCV, Amido Black and Hungarian Red. This did not meet up with the expectations. The expectations were that there was a difference in anility. Due to the fact that VMD is more sensitive to older samples (Masters & DeHaan, 1996). For the different surfaces, there was no statistically significant difference found between garbage bag, duct tape and filament tape. The addition of aluminium as a surface is recommended. But could not be further investigated in this study. For Hungarian Red on filament tape, it is recommended to take a lift to capture the fingerprint more easily. The lift should be taken with white gelatin lifter and lifted after leaving the lift on or over one hour (sixty minutes). The white gelatin lifter also has another advantage, the Hungarian Red will florescence with under infrared light (BVDA, 2020). Therefore, it is also recommended to investigate the lifts using infrared light for even better visualization of the print. There was no difference found in the detection limit of the samples after using VMD. The detection limit was the same for samples after treatment of VMD as with out VMD treatment.

This is one of the first studies to investigate VMD together with ALCV, Amido Black or Hungarian Red. For that reason, it is highly recommended to further investigate this subject. With more donors, higher number of samples and more surfaces. More donors and a higher number of samples will increase the confidence level. Using more surfaces, will broaden the knowledge of VMD reacting to surfaces, other than to glass or plastic substrates.

The findings in this study are renewing, because it did not meet with any expectations. Hopefully this study will be acknowledged in further investigations using VMD and combination traces. These findings will change the sequence in which a piece of conviction will be investigated.

6 Bibliography

Almog, J., Cantu, A. A., Champod, C., Kent, T., & Lennard, C. (2014). Guidelines for the assessment of fingermark detection techniques International Fingerprint Research. *Journal of Forensic Identification*.

Bodziak, W. J. (1995). The Use of Leuco Crystal Violet To Enhance Shoe Prints In Blood. *the European Meeting for Shoe Print and Tool Mark Examiners.* Helsinki: FBI Laboratory.

Bruton, A., Conway, J. H., & Holgate, S. T. (2000). Reliability: What is it, and how is it measured? *Physiotherapy*, 94-99.

BVDA. (2020, October 2). Retrieved from BVDA: http://www.bvda.com/NL/prdctinf/nl_lcv.html

BVDA. (2020, October 2). Retrieved from BVDA: bvda.com/nl/amido-black

BVDA. (2020, October 2). *BVDA.* Retrieved from http://www.bvda.com/nl/hongaars-rood-voor-schoensporen

Cummins, H., & Midlo, C. (1943). *Finger Prints, Palms and Soles: An Introduction to Dermatoglyphics.* New York: Dover.

Faulds, H. (1905). *Guide to finger-print identification.* Hanley: Oriel Works.

Fraser, J., Deacon, P., Bleay, S., & Bremner, D. H. (2014). A comparison of the use of vacuum metal deposition versus cyanoacrylate fuming for visualisation of fingermarks and grab impressions on fabrics. *Science and Justice*, 133-140.

Herschel, W. J. (1916). *The Origin of Finger-Printing.* London: Oxford University Press.

Jones, N., Mansour, D., Stoilovic, M., Lennard, C., & Roux, C. (2001). The influence of polymer type, print donor and age on the quality of fingerprints developed on plastic substrates using vacuum metal deposition. *Forensic Science International*, 167-177.

Jones, N., Stoilovic, M., Lennard, C., & Roux, C. (2001). Vacuum metal deposition: factors affecting normal and reverse development of latent ®ngerprints on polyethylene substrates. *Forensic Science International*, 73 - 88.

Kent, T. (1990). Recent research on superglue, vacuum metal deposition and fluorescence examination. *Police Scientific Development Branch (PSDB)*.

Kent, T. (2004). Manual of Fingerprint Development Techniques. In H. O. Branch. Sandridge.

Kent, T. (2013). Visualization or Development of Crime Scene Fingerprints. *Encyclopedia of Forensic Sciences*, 117-129.

Koo, T. K., & Li, M. Y. (2016). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of Chiropractic Medicine*, 155 - 163.

Langford, A., Dean, J., Reed, R., Holmes, D., Weyers, J., & Jones, A. (2005). Practical Skills in Forensic Science. Pearson Education Limited.

Lui, L., & Corma, A. (2018). Metal Catalysts for Heterogeneous Catalysis: From Single Atoms to Nanoclusters and Nanoparticles . *Chemical Reviews*, 4981 –5079.

Maltoni, D., Maio, D., Jain, A. K., & Prabhakar, S. (2009). Handbook of Fingerprint Recognition. Springer.

Masters, N. E., & DeHaan, J. D. (1996). Vacuum Metal Deposition and Cyanoacrylate Detection of Older Latent Prints. *Journal of Forensic Identification*, 32 - 45.

Mieloch, K., Munk, A., & Mihailescu, P. (2008). Hierarchically linked extended features in fingerprints. *Biometrics Symposium.* BSYM '08.

Misner, A. H. (1992). Latent Fingerprint Detection on Low Density Polyethylene Comparing Vacuum Metal Deposition to Cyanoacrylate Fuming and Fluorescence. *Journal of Forensic Identification*, 26 - 33.

Moenssens, A. A. (1971). Fingerprint Techniques. Chilton Book Company.

National Institute of Justice. (2004). The Fingerprint Sourcebook. Rockville: U.S. Department of Justice Office of Justice Programs.

NC Office of Indigent Defense Services. (2012, 09 17). *Technical Procedure for Amido Black.* Retrieved from Digital/Latent Evidence Section: http://www.ncids.com/forensic/labs/Latent/Technical/Amido-Black.pdf

Ramotowski, R. S. (2013). Lee and Gaensslen's Advances in Fingerprint Technology. CRS Press.

Schmidt, C. A. (1938). *The Chemistry of the Amino Acids and Proteins.* Springfield, Illinois: Charles C. Thomas.

Sears, V., Butcher, C., & Fitzgerald, L. A. (2005). Enhancement of Fingerprints in Blood, Part III: Reactive Techniques, Acid Yellow 7, and Process Sequences. *Journal of Forensic Identification*, 741–763.

Stroud, P. T. (1971). Some Comments on Finger Print Development by Vacuum Deposition. *Atomic Weapons Establishment (AWRE)*.

Suzuki, E. M. (1993). Forensic applications of infrared spectroscopy. In R. Saferstein, *Forensic Science Handbook* (pp. 196-248). New Jersey: Regents/Prentice Hall.

Theys, P., Turgis, Y., Lepareux, Z., Chevet, G., & Ceccaldi, P. F. (1968). *International Criminal Police Organization Journal*.

Thomas, G. L. (1978). The physics of fingerprints and their detection. *Measurement Science and Technology*, 722 - 73.

Zhang, D., Shen, Y., & Somorjai, G. (1992). Studies of surface structures and compositions of polyethylene and polypropylene by IR+ visible sum frequency vibrational spectroscopy. *Chemical Physics Letters*, 394-400.

Appendix

Appendix I – Protocol VMD

Table 8: Materials used in VMD chamber with corresponding manufacturer and details

Product	Manufacturer	Details
VMD chamber	Oerlikon Leybold Vacuum Nederland B.V.	UNIVEX 450 B Boiler content 100 L
Gold(wire) 99,99 %	Alfa Aesar GmbH & Co KG	Diameter 0,127 mm EC Number: 231-165-9
Zinc Granular	Merck KG	Particle size 3-8 mm EC Number: 231-175-3

1. Prepare all samples
2. Put samples in VMD chamber
3. Turn the hood on
4. Lower the pressure to < 3 x 10^{-4} mbar
5. Start gold deposition
6. Wait till filament reaches yellow-to-white heat
    a. Should be complete in 10 seconds
    b. Check if all gold is evaporated
        i. If not, increase temperature until all gold evaporated
7. Increase pressure in camber to ~5 x 10^{-4} mbar
8. Stark zinc deposition
9. Increase current until all filament glows in cherry-red to dull orange color.
10. Observe the whole process through the viewing window
11. Photograph all samples

Appendix II – Protocol ALCV

Formulation:
Two formulations involving hydrogen peroxide, sulphosalicylic acid, water and LCV have been used in the past with success. - The most recent formulation for the reagent, is as follows (Bodziak, 1995):

1. Dissolve 10 grams of 5-sulphosalicylic acid (Sigma # S-2130) in 500 ml of 3% Hydrogen Peroxide.
2. Add and dissolve 4.4 grams Sodium Acetate
3. Add and dissolve 1.1 gram of Leuco Crystal Violet (Sigma # L-5760) (A magnetic stirrer should be used)

(NOTE: If LCV crystals have become yellow instead of white, obtain fresh LCV)
After the solution is mixed it should be stored in dark colored glassware and refrigerated. It will last several months.

Application:
The above formulation for LCV includes 5-Sulphosalicylic Acid, which fixes the blood. therefore, the solution may be directly applied to the surface in one step.

This newer formulation appears to be more sensitive, results in a more vivid Violet color and eliminates some of the fading and color changes occasionally encountered with earlier formulations.
Applications of LCV may be made by lightly spraying (with an aerosol device) or by immersion. The reaction takes place rapidly. Where the LCV clear solution comes into contact with blood, the blood impression turns a purple/violet color, instantly providing improved visualization.

Appendix III – Protocol Hungarian Red

Fix prints in blood before staining
Before staining, prints in blood should be fixed to prevent them from running (causing loss of detail) when the staining solution is applied. Hungarian Red used, contains the fixation of 2% solution of sulfosalicylic acid.

Staining procedure
Once the print is fixed, it can be stained with Hungarian Red. Immersing in homogenous solution of the sample is recommended. To keep the solution homogenous, use a stirring flow.
Leave the sample for about one minute in the staining solution. Then wash the surface with rinse fluid.
It is advantageous to immediately remove the water and water droplets from the processed area after rinsing, with compressed air or a powerful blower. If water droplets remain on the print, you will notice that some dye will dissolve in them. After drying that will be visible (possible loss of detail).

Lifting and fluorescence
Hungarian Red not only has a good staining capacity but has also some special characteristics. Stained prints can be lifted with a white BVDA gelatin lifter so that backgrounds are eliminated and/or parts on dark backgrounds become visible. The lifted print will also fluoresce, whereby details or prints that were hardly visible with the naked eye are now easily discernible in fluorescence.

Before lifting with a white gelatin lifter, the area should be completely dry. Be very careful not to trap air bubbles under the lifter surface and leave the lifter on for 15-30 minutes. Photograph within a couple of hours after lifting (the dye will slowly diffuse in and across the surface of the lifter, thereby blurring the print). Lifting can be done more than once, with or without re-staining between lifts. Repeat lifting/staining in important cases. Fluorescence is excited with green light (515-560 nm) and the filter used in front of the lens is a red filter (Kodak Wratten 25, a barrier filter of about 600 nm or a long-pass filter with cut-on of about 600 nm) (BVDA, 2020).

Appendix IV – Protocol Amido Black

Materials
- Protective gloves and apron/coat
- Face shield visor and/or safety goggles
- Magnetic stirrer, magnetic follower, and magnetic retriever
- Two (2) 2000 mL glass beakers
- One (1) 1000 mL glass beaker
- Dark, shatter-proof container

Reagents
- 5-Sulfosalicyclic acid (20 g)

- Naphthalene 12B, Napthol Blue Black, or Amido Black (3 g)
- Sodium carbonate (3 g)
- Formic acid (50 mL)
- Acetic acid (50 mL)
- Kodak Photo Flo 600 Solution (12.5 mL)
- Distilled water

Preparation

1. Pour five hundred (500) mL of distilled water into a clean two thousand (2000) mL glass beaker and place on a magnetic stirrer.
2. Place a magnetic follower in the distilled water and stir while adding the following in order:
   - 5-Sulfosalicyclic acid (20 g)
   - Napththalene 12B, Napthol Blue Black, or Amido Black (3 g)
   - Sodium carbonate (3 g)
   - Formic acid (50 mL)
   - Acetic acid (50 mL)
   - Kodak Photo Flo 600 Solution (12.5 mL)
3. Stir until all crystals are dissolved.
4. Dilute mixture to one (1) liter with distilled water.

Amido Black solution may be kept indefinitely in a dark, shatter proof container.

Application

1. Dip the samples in the staining solution.
2. Completely cover the targeted area with staining solution
3. Allow to develop 3 – 5 minutes.
4. Rinse with flushing fluid to remove any excess staining.

Appendix V – Fingerprint samples

Table 9: Amount of samples

Sample	*Amount samples*	*Notes*
Fingerprints	108	36: 24 hours old 36: 2 weeks old 36: 24 hours old (No VMD)
Dilutions	27	9: 1:100 9: 1:500 9: 1:1000
Surfaces	120	24 – Glass 24 – Garbage Bag 24 – Duct tape 24 – Filament tape 24 – Aluminum
Total samples	***255***	

Appendix VI – Plan of approach

"Influences of VMD on ALCV, Hungarian Red & Amido Black"

1. Introduction

A fingerprint is a copy of the characteristic outward appearance of the fingertip epidermis. Or as Edmond Locard said, *"Every contact leaves a trace",* in this case a fingerprint. Johann Mayer made the first detailed description of the anatomical formation of fingerprints in 1788. He wrote that friction ridge skin is unique (Moenssens, 1971) (Cummins & Midlo, 1943). Sir William James Herschel was the first person to study persistence of friction ridges (Herschel, 1916). In 1880, Henry Faulds was first to publish in a scientific journal about the value of ridge skin for individualization. Faulds published in 1905 a guide to Finger-print identification (Faulds, 1905). In 1898, a criminal case in Bengal is considered the first case in which fingerprint evidence was used to secure a conviction. In the first cases where fingerprints were used as evidence, the prints were manually indexed and matched. More and more cases came in to be identified and the manual method became overwhelming. This led to the development of Automatic Fingerprint Identification Systems (AFIS), which is still used nowadays (Maltoni, Maio, Jain, & Prabhakar, 2009).

Fingerprints are mainly distinguishable in tree forms. Patent prints (visible prints), latent prints, (not visible with naked eye) and plastic prints (formed in/on a substrate) (Langford, et al., 2005). Two (or more) prints can be matched by characteristic features, generally in three levels (Figure 1). Level 1 features are macro details or overall ridge patterns such as ridge flow and pattern type. Level 2 features refer to minutiae (Galton details) such as ridge bifurcations and endings. Level 3 features include all dimensional attributes of the ridge such as width, edge contour, pores, shape, scars and other permanent details (Mieloch, Munk, & Mihailescu, 2008).

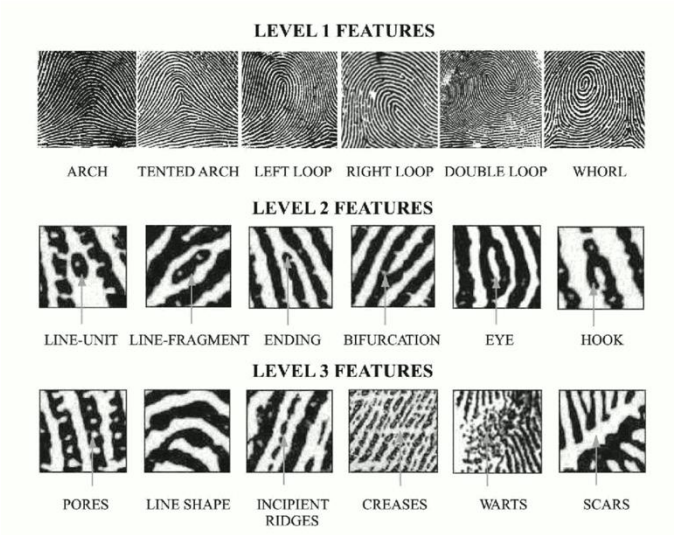


Figure 17: Three different levels features. Level 1 features are overall ridge patterns. Level 2 features are the minutiae. Level 3 features are all dimensional attributes (Mieloch, Munk, & Mihailescu, 2008).

Various methods and approaches can be used to recover a fingerprint depending on the type of print. The simplest approach to visualize latent fingerprints is powder dusting. The method relies on the mechanical adherence of the powder to the sweat components of the skin deposits. Another method is chemical fuming. One example and the most common one is cyanoacrylate (Superglue™) fuming. This method uses the deposition of polymerized cyanoacrylate ester onto the latent fingermark residue to develop the print (Ramotowski, 2013).

Vacuum metal deposition

Vacuum metal deposition (VMD) is used to develop latent prints involving a coating/depositing a thin layer of gold and zinc onto a surface by evaporation by thermal sublimation under vacuum. First a thin layer of gold is deposited, the gold diffuses on the fatty residues of the latent fingerprints. Zinc will not hence on fatty residues (even if the residues are underneath the layer of gold), but it will deposit on small nuclei of metal. After the thin layer of gold, a layer of zinc is deposited, it will condense on the spots where there is no fatty residue underneath (Figure 2) (National Institute of Justice, 2004) (Stroud, 1971).

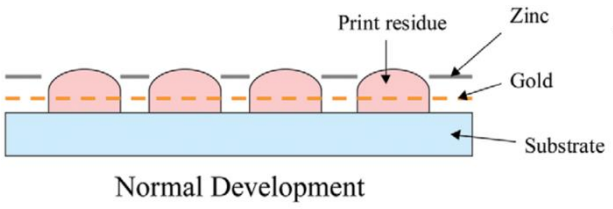


Figure 18: Schematic view of vacuum metal deposition. (National Institute of Justice, 2004)

Both VMD and cyano fluming are effective methods of visualizing latent prints and have much overlap. But there is one difference, VMD had the advantage that it can develop fingermarks on objects that have been wet or ages, whereas cyano fuming does not (National Institute of Justice, 2004). When investigating both methods on fabrics, VMD came out five times more likely to provide marks to include or exclude an individual (Fraser, Deacon, Bleay, & Bremner, 2014).

Staining solutions

Blood is known as one of the most common contaminants of fingerprints found at crime scenes (National Institute of Justice, 2004). Some fingerprints are (in)visible, some prints are underneath blood and some prints are made in blood or combinations. In these combinations, parts of the fingerprint are latent and parts are made in blood. To visualize all the prints blood enhancement techniques are used. In this research, the three main techniques are used: ALCV, Hungarian red and amido black.

ALCV

ALCV (Aqua Leuco Crystal Violet) is a colorless to pale blue aqueous solution. The reagent can be used in two ways. It can be sprayed on the surface or immersed in the solution. When it comes into contact with blood, a catalytic reaction takes place under the influence of the hemoglobin in which the ALCV is oxidized by the hydrogen peroxide to the strong purple dye crystal violet. If necessary, rinse with demineralized water to rinse away unused reagent. Advantages are that in principle there is no need for rinsing, it works on both porous and non-porous surfaces, the trace does not need to be fixed, the reagent already contains fixer, it does not contain volatile solvents and is chemically safe (BVDA, 2020).

Hungarian Red (Fuchsin Red)

Hungarian Red or Fuchsin Red is a water-based dye for (traces of) blood. Because it is water-based it is a safe agent, it colors well and can be lifted with white gelatin foil, which is a good advance. When lifted with white gelatin foil the print can be investigated with green light and will fluoresces on the foil. In this way it is possible to secure even weak tracks placed on dark surfaces (BVDA, 2020).

Amido black

Amido Black is a coloring agent that can color proteins, as present in blood, blue-black. Amido Black is supplied on a methanol basis as well as on a water and water / ethanol / acetic acid basis. Amido Black in methanol has a stronger color but is also more dangerous due to the toxicity of the methanol (BVDA, 2020).

Issue

Following case was in the Dutch papers: As a statement in the criminal circuit to an enemy, a severed head of a victim was placed in a garbage bag on the street in front of a shisha lounge in Amsterdam

Southeast. The bag was found on an early morning in March 2016 with a temperature of ±7.5 °C. Obviously, the garbage bag will contain a lot of blood, but also perpetrator traces such as fingerprints.

In the fictional case above some main problems arise concerning fingerprint recovery:

- The elapsed time from making the print to discovering the print
- The nature of the surface on which the print is present
- The method(s) to recover, sequencing.

When an object had been wet due to any kind of factor, cyano fuming is not possible anymore. VMD is an alternative to cyano fuming. But VMD can have influences on bloodstaining methods, which are not investigated yet. Staining solutions and reagents used for improving the contrast of marks (fingerprints and footmarks) made in blood. This issue can be examined by the main question: What are the influences of VMD on blood enhancement techniques?
Following sub-questions arise with the main question:

- To which dilution factor of blood does the blood enhancement technique still work?
- Is there a difference in the three blood enhancement techniques?
    - What are those differences?
    - Do protein stains work better than hemoglobin stains?
- Is it possible to lift a print after using Hungarian Red with white gelatin?
- Is it possible to enhance blood with Hungarian red, ALCV or amido black after VMD?
    - In the extent to which does VMD stain blood?
        - Is it still necessary to use staining solutions?
- What influence does the anility of the fingerprint have?
    - On VMD
    - On bloodstaining solutions
- What blood enhancement technique can be used best on what surface material?

2. Approach

To investigate the influences of VMD on bloodstaining solutions, the following approach will be executed. The three main bloodstaining methods used in this study are Hungarian Red, Amido Black and ALCV. To investigate the influence of VMD on blood enhancement techniques, different slides will be provided with a print, treated with VMD and stained with blood enhancement techniques. All the samples will be compared to one another in duplo with a negative control. All samples will be made by three different donors and equally divided.

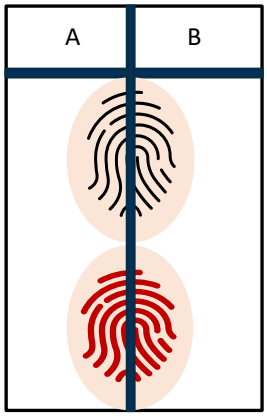


Figure 19: Two object slides (A and B). With 2 fingerprints, Blue print is regular fingerprint. Red print is a fingerprint made in blood.

Preparing fingerprints

Fingerprints will be made by two donors. To create the best duplo's, one fingerprint will be put onto two slides at the same time, in this way halve of a print is on one side of the microscopic slide. For the negative control, one fingerprint will be placed with blood (Figure 8, red fingerprint) and one fingerprint will be placed regularly without blood (Figure 8, blue fingerprint).

Anility

For the anility/aging of the samples, samples will be prepared 24 hours and 2 weeks before examination. The average time during which a piece of conviction is examined is around 2 weeks.

VMD

All microscopic slides will be put in VMD hood. And will be developed following the instructions of the manufacturer. The methods and equipment used may vary according to the manufacturer. But essentially the elements are the same. The equipment consists of a vacuum chamber capable of being pumped down to very low pressure (< 3 x 10^{-4} mbar), filaments for evaporation of gold and zinc and a window to monitor the deposition of the metals. Some chambers may also contain a "cold finger". This finger helps reduce pump downtimes due to its low chilled temperature. Gold deposition takes place when the pressure in the camber has reached 3 x 10 10^{-4} mbar or lower, and the current to the filament is increased until the filament reaches a yellow-to-white heat. The deposition of gold should be complete within 10 seconds, but when all gold is not evaporated, the temperature should be increased until all residue is gone. After the golf deposition, the pressure in the camber is increased to ~5 x 10^{-4} mbar and the current to the zinc deposition is turned on. Increasing the pressure in the camber is to reduce the speed of zinc deposition by introducing more air molecules with which the zinc may collide. Zinc deposition filaments are larger and deeper than the gold filament, and the quantity of zinc added is greater, 1 gram per run. For zinc deposition, the current is increased until the filament glows a cherry-red to dull orange color. This process should be observed by the operator trough the viewing window. After zinc deposition, the gold filaments should be heated shortly to yellow-to white heat to burn off zinc contamination (Kent, Manual of Fingerprint Development Techniques, 2004) (National Institute of Justice, 2004). After VMD all microscopic slides will be photographed.

Blood enhancement techniques

Different slides will be developed by different blood enhancement techniques. 15 samples (with duplo) will be developed using one of the three blood enhancement techniques.

The solution for ALCV contains the fixation for blood. Therefore, the solution can be applied directly to the samples. The application may be made by lightly spraying or immersion of the samples. The reaction takes place fast. The places where ALCV solution makes contact with blood, the blood impression tuns a purple to violet color.
For Hungarian Red, the samples need to be fixated first, with a 2%-sulfosalicylic acid solution and an absorbent paper. After fixation the samples can be staining using the staining solution and a spray bottle. Leave the staining solution on the samples for about one minute. Then wash of the solution with water or a 5% water/acetic acid mixture = 19:1 volume/volume ratio).
Amido Black may be applied to the samples either by using a spray bottle or by dipping the samples in the staining solution. Completely cover the target area and allow to develop for approximately three to five minutes. Rinse samples with tap water to remove any excess reagent after development (NC Office of Indigent Defense Services, 2012).

Blood dilutions

Different blood dilutions onto one microscopic slide, the microscopic slides contain dimples. In this way the dilutions will be made in triplo. 25 µl of dilution will be put in one dimple. 9 samples will be made for each concentration of 1:100, 1:500 and 1:1000, 27 samples in total. For the negative control, water will be used. In Figure 4 an example of a microscopic slide is shown. The samples will be dried for at least 24 hours.

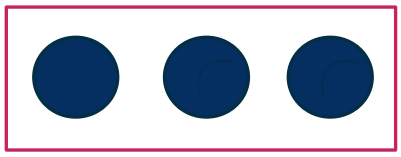


Figure 20: A microscope glass with 3 dimples. In every dimple the same dilution of blood is added. In this way the samples are made in triplo.

Surfaces

For the research on different surfaces the following surfaces will be used:
glass, garbage bag, duct tape, armored tape and aluminum. For each surface 40 samples will be taken. The samples will be taken in the same way as Figure 8.

Scoring

10 different assessors will score the fingerprints following table x. This scaling table was made by Centre for Applied Science & Technology (CAST) (Almog, Cantu, Champod, Kent, & Lennard, 2014)

Table 10: Grading system for the assessment of fingermark detection techniques International Fingerprint Research Group (IFRG) (Almog, Cantu, Champod, Kent, & Lennard, 2014).

Grade	Details
0	No development
1	Signs of contact but < 1/3 of mark with continuous ridges
2	1/3–2/3 of mark with continuous ridges
3	> 2/3 of mark with continuous ridges, but not quite a perfect mark
4	Full development – whole mark clear with continuous ridges

Validation

An ICC-test will be performed on all data to determine whether the different assessors agree with one another and whether the data to be used is reliable. Since the fingerprints are placed in the center of two microscopic slides, it can be reasonably assumed that the two halves of one print have more similarities than two random halves. For this reason, a paired test will be used. Since the data is assumed to not normally be distributed, because a shift from the mean is expected, Mann-Whitney U test will be used. After the statistical tests have been performed, the data will be presented in a table or bar chart with error bars, depending on the amount of data.

3. Timeline

Task	**Date (2020/2021)**
Basic bloodstain analysis cursus	7 – 11 September
Writing plan of approach Preliminary investigation	14 September – 2 October
Hand in PoA	12 October
Research	12 – 30 October
Correct PoA PoA convert to report	2 – 6 November
Make a presentation Research	9 – 13 November
“TerugKomDag”	20 November
Research	16 – 19 November 23 November – 22 December
Completion of research Completion concept	28 December – 2 January
Hand in concept at Loci	13 January
Correct concept	19 January – 22 January
Hand-in concept school	22 January
Correct concept to final report	5 – 10 february
Hand-in final report	10 february
Final presentation	24 february

4. Fingerprint samples

Table 11: Amount of samples

Sample	*Amount samples*	*Notes*
Fingerprints	60 (x 2)	30: 24 hours old 30: 2 weeks old
Dilutions	27	9: 1:100 9: 1:500 9: 1:1000
Surfaces	60	10 – Glass 10 – Garbage Bag 10 – Ducttape 10 – Armored tape 10 – Aluminum
Total samples	***207***	

5. Domain Applied Science Competences

Investigate level III	
The student translates a problem into a research strategy and carries out the research.	*I will show that through*
Implement the work plan effectively and efficiently and adjust it if necessary.	Set up an effective and efficient work plan with a panning prior to the research The work plan will be adjusted in the meantime, if necessary. Some extra time is also planned in the work plan for any delay, if necessary.
To report on the research in accordance with the standard applicable in the professional field.	The final investigation will be published in a forensic journal. Therefore the final report will be converted into a scientific article.
Formulate a strategy for follow-up research; to make a proposal for next steps based on the analysis of the results.	The research area in the combination as I will conduct it has never been published. Therefore, the research will be the base for this method and all follow-up studies. Less different factors are included, so it is up to follow-up research to include these other factors.

Experimenting level III	
the student sets up experiments with supervision and carries them out independently and systematically.	*I will show that through*
To take into account the possibilities and limitations of the equipment to be used when designing and conducting experiments.	Vacuum metal deposition will be used. This method has many possibilities that have not yet been properly explored. That is why this must be further investigated and included in the investigation. VMD chamber will also have to be calibrated.

Choosing a (statistical) method to assess the reliability of the result found.	The reliability of the results in this study will be statistically substantiated. Intraclass correlation and Mann-Whitney U test will be used.
Make a schedule for the design and execution of experimental work within a project of longer duration (at least six months, such as for the graduation assignment), requiring regular adjustment of the schedule based on progress.	Draw up a time schedual and include it in the PoA for a period of approximately 5 months. Adjust the planning in the meantime if necessary.

Developping level III	
The student develops or improves by independently drawing up an approach.	*I will show that through*
Select the most appropriate subject-specific design parameters that can influence the process, product, instrument or material.	Searching in the literature for design parameters and selecting them, in order to lay the foundation for the VMD method. Include the influences of these parameters in the method.
To draw up the documentation of the development and the result according to the standard applicable in the field.	The documentation of the research will eventually be converted into a scientific article, which meets the standard applicable in the field. The FO standards are also included in the method, in connection with, for example, the influences of the transport of tracks.

Managing | Coordination level II	
The student contributes to one or more management systems within the organization.	*I will show that through*
Making proposals to solve problems that arise during the implementation and maintenance of a management system.	In case of problems I encounter, consult with the supervisor. And making proposals for solutions and / or maintenance. This with regard to the VMD device that I will calibrate and develop a method for.
To fit the performance of his work into the management systems used at his workplace.	The documentation of the research will eventually be converted into a scientific article, which meets the standard applicable in the field. The FO standards are also included in the method, in connection with, for example, the influences of the transport of tracks.

Advise | Buying and selling level III	
The student gives concrete advice on a specific question.	*I will show that through*
To come up with creative solutions to specific problems or developments.	Solution-oriented thinking, for some problems a creative and simple solution is THE solution! Thinking out of the box. (My supervisor is the best at this so I will learn a lot from him).
Taking into account the customer's environment.	In my case, the customer or client will partly be the police. I have to take this into account. With

	the working environment they have there and observe the associated rules

Instructing | Guiding | Teaching | Coaching level I	
Upon request, the student passes on his / her own knowledge and skills to employees (by demonstrating and explaining).	*I will show that through*
To contribute to the instruction / demonstration to fellow employees, students or pupils regarding a practical test, etc.	When a course or workshop is given on location, I can contribute by helping or guiding (I have already obtained a basic bloodstain analysis certificate myself).
To be aware of the importance of continuous expertise development.	I am aware of the importance of the continuous development of expertise. My supervisor is an expert and can tell me all about this.

Leadership | Managing level I	
The student provides assistance and gives direction to employees when requested.	*I will show that through*
Act honestly and reliably towards employees, fellow students and teachers.	Part of the investigation is confidential, so it goes without saying that reliability is very important here. Honesty is also very important within this team. That you know where you stand, what you can expect from each other.

Self-management level III	
The student guides himself in his own functioning.	*I will show that through*
Adapt its functioning to the requirements of the different working environments.	On location at loci there will be certain requirements in the work environment that I will meet. But also with the police, that environment will be very different. I will have to adjust my performance accordingly.
Adjust your own functioning on the basis of experiences.	Asking for areas for improvement and willing to improve. When something goes wrong, also know why it went wrong and how this can be prevented the next time. These are the right learning moments.
To justify one's own actions to others and to motivate choices to be made.	To justify one's own actions to others and to motivate choices to be made.

Appendix VII – Judgment and Sustainable Development

People, planet and profit were taken into account in this research or Judgment and Sustainable Development. **P**eople (the human side): attention to people, employees and discussion partners. **P**lanet (environment and energy): attention to the environment, society, raw materials and alternatives, now and in the future. **P**rofit (the financial side): attention to the market and the necessity of profit and continuity for a company.

Using gloves during the experiments will follow in a successful experiment. In this way, any contamination is excluded and failing of the experiments is less likely to happen. Resulting in less materials used (profit and planet). Using gloves can also protect the user from the dyes used, just like using a coverall (people). The coveralls are used again during experiments unless they are not safe to work with anymore (blood on the coverall or any damage) (profit and planet). During the sampling of the fingerprints onto the microscopic slides, all the space is being used. Even one microscope glass is being used for the sample and at the same time for a control. This results in less usage of microscopic slides and is in that may more sustainable (profit). When a sample (or experiment fails/ends) the microscopic slides are being reused after being cleaned in an ultrasonic bath (planet and profit).
Looking at the aim of the study, it might be possible that the blood enhancement methods are no longer necessary, that VMD is enough to stain all latent marks. Which results in using less chemicals in the field (planet). Another important part is the part where the samples are immersed in the blood enhancement solution. In this way less solution is used than when spraying the solution on the samples(profit). ALCV, Amido Black and Hungarian Red used in this study were water based, so no harmful chemicals were used (people and planet).